

## Some Observations on Gape-worm in Poultry and Game Birds.

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### INTRODUCTION.

IN the literature which deals with the gape-worm, *Syngamus trachea*, found commonly in England, its presence has been recorded from a fair variety of birds. It has been found several times in the starling—Nathusius (1837), Megnin (1883) and, among others, by Lewis (1925). Dujardin records having found it in the magpie, *Corvus pica*, (1845); Megnin (1883) found it in the swift (*Cypselus apus*), green woodpecker (*Picus viridis*), black stork (*Ciconia nigra*) and in the pheasant and partridge. It has further been recovered by Lewis (1925) from the rook (*Corvus frugilegus*), thrush (*Turdus musicus*) and jay (*Garrulus glandarius*), while in the Ministry of Agriculture Leaflet No. 58, the sparrow and linnet are also cited as being occasional hosts, and Walker (1886) has found it in the robin. The present writers have found a closely related species, *S. merulae* (Baylis, 1926) in the tracheae of four blackbirds which came into the laboratory during the summer. Though these birds seem to be only occasional hosts, most of the authors stating that the infections occurred only in young birds while the adults are comparatively free from this worm, yet a high percentage of young birds are frequently found to be carrying this parasite : for instance, Elton and Buckland (1928) in a batch of 33 young rooks obtained from the Oxford district, found 31 infected, while of 8 old birds only four were infected and these only lightly. Lewis in 1926 found 35 per cent. of a sample of starlings infected but he did not notice the age of the birds. Morgan examined two nests of young starlings and found that all of one batch harboured *S. trachea*. He suggests that they may have become infected from the earthworms brought in by the parent birds as food.

Taylor (1928) has pointed out that eggs obtained from starling gape-worms have only a low infectivity to young chickens. Once an infection has been obtained, however, the second generation of eggs enters the chicken readily. He puts forward the theory that though the gape-worm from the starling is morphologically indistinguishable from that of the chicken and turkey, yet it is a distinct physiological strain and he therefore concludes that, from the point of view of carrying "gapes," the starling is harmless to the chicken.

#### MATERIAL.

Through the co-operation of Mr. A. D. Middleton of the Bureau of Animal Population, Oxford University Museum, to whom we are much indebted, we were able to obtain a considerable number of birds of many kinds from a large number of estates from all over England and from these we were able to collect gape-worms. Of these birds, *S. trachea* was recovered from the partridge, pheasant, rook and starling, while *S. merulae* was recovered from the blackbird.

Two batches of young rooks, of this year's hatching, were obtained from the Longwood Estate, near Winchester, and a 100 per cent. infection of *S. trachea* was found, most of the birds being heavily infected and containing 30 or more pairs of worms in the trachea. A private note from Mr. Middleton tells us also of a heavy mortality in young rooks which occurred during the first and second weeks of June at Wherwell Priory, Hants. These birds all harboured a heavy infection of gapeworms which was diagnosed as being the cause of death. Many young partridges and young pheasants were obtained from various estates and in a large number of cases *S. trachea* was found to have been the lethal factor. In view of these findings and of the fact that the gape-worm has been recorded from several wild birds, it would seem that this disease is a very important factor in controlling the numbers of many species of birds and, furthermore, that several wild birds may act as reservoir hosts and may serve to transmit the disease, particularly to young poultry and game birds in the spring of each year.

In the experiments described below the chicken, pheasant, turkey and partridge were used as experimental animals. The chickens were Rhode Island Reds  $\times$  Light Sussex first cross, cockerels, incubator hatched, and they were reared under parasite-free conditions in batteries indoors. The

turkey poult were also incubator hatched but they were reared under clean hens outside on ground which had not carried any birds for many years. A few which died before or during the experiments never contained any helminths. The partridges and pheasants were obtained through the assistance of the Bureau of Animal Population. Most came as day-old birds and were reared under hens as were the turkeys. Of one covey of pheasants which arrived when a fortnight old, a few already harboured gapes.

#### TECHNIQUE.

The gape-worms were removed from the trachea by dissection of the infected birds which came into the laboratory. They were teased up and as far as possible, the gut and anterior portion of the worms were removed so as to keep the cultures clean. The eggs, most of which were then free in the water, were cultivated in shallow petri dishes on the bench in the laboratory. They were well aerated daily and it was usually found that the infective stage was reached in about 10 days. Generally, however, the eggs were not fed to the birds until later, allowing plenty of time for complete development to the infective stage to be reached. At the time of feeding, the larvae could generally be seen actively moving inside the shell ; some larvae had already hatched.

The method of feeding was as follows :—As much fluid as possible was drawn off from the culture, thus concentrating the eggs. A small drop of this concentrated fluid was removed by means of a fine pipette to a slide and all the infective eggs counted. If more eggs than were wanted were present, a number were removed with a piece of filter paper ; if there were too few, then more were added. When the required number was isolated on the slide, a small quantity of flour was added slowly and mixed with the water until a small pellet containing the eggs was formed. This was fed to the chicks. By this method, the size of the dose could be accurately controlled and it is estimated that the error was never more than 5%.

#### EXPERIMENTS.

##### A. Material from the Rook.

A quantity of gape-worms identified as *S. trachea* was obtained from rooks (*Corvus frugilegus*). The results of feeding these eggs to chickens were watched with great interest as Rice (1929) found that the administration of gapeworm eggs from rooks caused a 100% infestation in chickens.

Typical symptoms of "gapes" were noticed 9 days after feeding and he infers therefore that the rook is an important potential cause of loss to poultry keepers.

EXPERIMENT 1.—Ten chickens, aged two days, were fed with small doses of infective eggs of rook origin. The doses were 10, 20, 30, etc., up to 100. No symptoms of the disease were seen and at autopsy four weeks later, no gapes were found. The experiment gave completely negative results.

EXPERIMENT 2.—Ten chicks aged nine days were each fed 50 infective eggs from the same cultures. Two birds died accidentally 13 days later and each contained four pairs of gapes in the trachea. The others were autopsied later but no worms were found.

EXPERIMENT 3.—Seven turkey poult were fed infective *Syngamus* eggs of rook origin, in doses varying from 20 to 150 eggs, when the birds were thirteen days old. Bird No. 37, which had been fed 80 infective eggs, died from causes other than gape-worm infestation. It contained three pairs of well developed worms, placed high up in the trachea near the mouth. All the other poult survived and showed eggs in the faeces when examined 6 weeks after feeding ; eggs are still appearing after 16 weeks. A group of 10 poult which were running on an adjacent plot of land acted as controls. They have showed no evidence of having picked up the disease from the land. Three which died contained no worms and none of them have shown any eggs in the faeces.

EXPERIMENT 4.—Seven young pheasants, 14 days old, were sent to the laboratory and within a day or two of their arrival they were fed with eggs of *Syngamus* of rook origin, in doses varying from 50 to 400 eggs. Faecal examinations were unfortunately not made before the infestation. Nine days after the experimental feeding one died from exposure and post-mortem examination showed the presence of a pair of gape-worms lodged at the base of the trachea. These worms were very large and could not have been the result of the experimental feeding, but there were also 7 pairs of very small gape-worms which might easily have been the result of the experimental feed. Faecal examinations of the remaining birds were made 3 and 5 days later and 5 others were found to be lightly infected. It is impossible from this experiment to say definitely that the rook strain of *Syngamus trachea* is infective to pheasants though the presence of the

small forms is circumstantial evidence. Furthermore, three weeks later, it was observed that three birds had begun to "gape" very noticeably, a symptom which had not been observed in any of the covey previously. These birds gaped for about three weeks and then ceased. Faecal examinations, made 9 weeks after the experimental feed, *i.e.*, when the birds were about 12 weeks old, showed no eggs, suggesting that, the whole infection had been thrown off.

**EXPERIMENT 5.**—As somewhat disappointing results had been obtained in transmitting this rook material to chickens, it was decided to feed enormous doses as Rice had done. For this purpose, a very large number of eggs was concentrated in a small quantity of water and these were fed to four chicks aged three weeks, partly in pellets and partly directly in water by means of a fine pipette. These chicks remained entirely negative for gapes. Later, two chicks, aged 24 days, were fed with similar doses. Five days later one died and on autopsy very severe lung lesions caused by second and third stage *Syngamus* larvae were seen and these had obviously been the cause of death. The other chick was killed 18 days after feeding and one pair of small gape-worms were found in the trachea ; the lungs were normal. The actual number of eggs administered was not known but it was estimated that the dose was at least 10,000.

From these results it would seem that the rook may be a definite source of danger as a carrier of gape-worm. This strain is particularly infective to turkey pouls and probably to young pheasants also, while only variable results have been obtained by the present writers with chickens.

#### B. Material from the Pheasant.

This material was identified as *Syngamus trachea*. Four chicks, aged three weeks, were each fed 100 infective eggs. At autopsy, 22 days later, two chicks were negative while each of the other two harboured one pair of gapes in the trachea.

#### C. Material from the Blackbird.

A small number of gape-worms, identified as *Syngamus merulae*, were obtained from the blackbird. Infective eggs were fed in doses of approximately 50 eggs to two chicks but in no case did an infection result.

#### D. Material from the Partridge.

This material, also identified as *S. trachea*, was fed to six chickens but no infections resulted from these feeds.

## EARTHWORM EXPERIMENTS.

It has been known for some time that earthworms may carry the eggs of *Syngamus*. This must have been the case in the nestling starlings examined by Morgan in 1931. As he points out, starlings are remarkably clean in their nesting habits and practically the only source of infection of "gapes" was the food, which consists largely of earthworms. But that earthworms are not obligate intermediate hosts is also obvious as positive results have often been obtained by direct feeding with eggs.

It was determined to test the importance of earthworms as vectors. For this then, a large quantity of earthworms—probably *Lumbricus terrestris* and *Eisenia foetida* were obtained from a small copse. No domestic bird had ever been there. It was therefore considered that the earthworms were unlikely to carry a natural infection of *Syngamus*. These worms were washed in water and placed in blotting paper until the gut was completely emptied of soil. They were then transferred to clean soil which had been sterilised by steaming for one hour, mixed with some sterile damp peat moss. Generally they thrived in this medium though on some occasions many of them died, the reason not being obvious. When they had become acclimatised to this medium, *Syngamus* eggs from a known host were added in a small quantity of water. The worms were allowed to remain in contact with this infected soil for at least three weeks, during which time all the soil seemed to pass through the gut. Before feeding the alleged infected earthworms, they were washed and cleaned with a small brush in order to remove any eggs that might be adhering to the mucus on the surface of the body.

An observation which suggested the potential use of earthworms was made in a single experiment carried out early in the course of this work. Earthworms had been infected with material from the rook and five such worms were fed to each of four chickens, aged two weeks. Ten days later, one chicken—No. 38—was observed to be gaping and two days later, a second chick—No. 37—also began to gape. All the chicks were killed 22 days after feeding. The post-mortem examination showed that No. 38 contained 18 pairs of *Syngamus*; No. 37 contained 7 pairs, while Nos. 32 and 31 contained two and three pairs respectively. All these worms were attached in the upper third of the trachea; they were well developed, the average length being over 2 cms., and were passing eggs.

This condition was investigated more fully and further results will be published shortly by one of us. Earthworms identified as *Lumbricus terrestris* and *Eisenia foetida* were placed in sterile soil containing eggs from rook, pheasant, partridge and starling.

#### EXPERIMENT 1. *Rook Material.*

Four chicks were fed : two received four *Lumbricus terrestris* and the others had four *Eisenia foetida*. There were four control birds, each of which was fed a direct infection of 200 infective eggs. The two birds which had *Eisenia foetida* developed "gapes" and harboured 7 and 9 pairs of gape-worms respectively in the trachea. None of the others became infected.

#### EXPERIMENT 2. *Pheasant material.*

Earthworms were similarly infected with gape-worm eggs of pheasant origin. Eight chicks, aged eight days, were used in this experiment also. Nos. 9 and 14 were each fed two *Eisenia foetida*; Nos. 13 and 14 each had two *L. terrestris* which had been in the same infected soil as had the *E. foetida*. The four control birds each had 100 infective eggs in a direct feeding. Eighteen days after the experimental feed Nos. 9 and 14 were seen to be gaping. All the birds were killed after 28 days. No. 9 contained two pairs of very large gape-worms and No. 14 contained one pair, all attached to the upper half of the trachea. All the other birds were negative.

#### EXPERIMENT 3. *Starling material.*

Six chicks, aged nine days, were used in this experiment. Three of them received *Eisenia foetida* which had been in contact with eggs from gapes of starling origin, and the other three received a direct infection of 100 infective eggs. None became infected with "gapes." We possessed, however, only a very small quantity of material. It is doubtful if the earthworms could have been at all heavily infected.

#### EXPERIMENT 4. *Partridge material.*

Eight chicks were used in this experiment, which was arranged as the one involving the pheasant material. No infections resulted from these feeds but one point may be mentioned which may explain the negative results. Some difficulty was experienced in obtaining earthworms owing to a long spell of dry weather. Eventually sufficient were obtained but they were in a sluggish condition, lying curled up and dormant at the end

of their burrows. They remained alive in the jar containing the infected soil for two days only and hence it is unlikely that they had had time to become infected. They were all *Eisenia foetida*.

#### CONCLUSIONS.

*Syngamus trachea* has been obtained from the rook and pheasant, in both of which there were very heavy infestations and from the starling and partridge, in which the infestations were lighter. Infections to chickens were attempted from all these sources with varying degrees of success. A great variety of doses were given but only occasional successes can be recorded with the rook and pheasant material, while wholly negative results were obtained with the partridge and starling material. No infections were obtained from feeding eggs of *S. merulae* from black-birds to chickens.

Much greater success has, however, been obtained by feeding earthworms with eggs of *S. trachea* and then feeding these earthworms to chickens. It is not merely that the gape-worm has been passed to chickens, but the actual disease, with all its attendant symptoms, has been produced.

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## Some Observations on the Response of Chickens to infestation with *Heterakis gallinae*.

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Experimental evidence of immunity to helminth parasites is on the whole very scanty. Several workers have drawn attention to the fact that immunity can be acquired as a result of age alone. Ransom (1921) using *Syngamus trachealis* in chickens, Herrick (1925) with *Ascaridia lineata* also in chickens, Sandground (1928) with *Strongyloides* and *Nanophyes* in dogs and cats, are three examples.

There are also observations that immunity can be acquired as a result of a previous infestation, quite apart from age. In this respect the references are more numerous. *Ancylostoma caninum* in dogs and cats has been considered by Sarles (1929) and McCoy (1931), *Haemonchus contortus* in sheep by Stoll (1929) and, to turn to the trematodes, *Schistosoma japonica* in dogs and cats by Ozawa (1930). They all obtained evidence that having once been infected re-infection can occur, but the symptoms are milder, the percentage survival of the parasites less and their growth retarded. The immunity is not absolute as in the case recorded by Blacklock and Gordon (1927) who used larvae of *Cordylobia anthropophaga* (Diptera) on the guinea pig.

On the other hand McCoy found no such acquisition of immunity when he used two species of *Hamacreadium*, which are fish trematodes. Cestodes seem to have been but little considered in this respect. There are some isolated observations, but experimental evidence is singularly lacking. Miller in a series of papers from 1929 onwards has obtained positive immunisation against cestodes, using *Taenia taeniaeformis* and its larval form *Cysticercus fasciolaris* in albino rats. He made intraperitoneal and subcutaneous injections of powdered worm material and checked the development of the larvae in the liver. It is interesting to note, however, that so far he has been completely unsuccessful in the converse experiment. Powdered material from either adult worms or larvae is ineffective in preventing the growth and development of the adult worm in the

intestine of the cat. His results depend, however, on the serological reactions which are not being considered here.

The effect of diet is also excluded, as it has already been considered by the present writer in a previous paper (1932).

*Heterakis gallinae* in chickens was used for this work, for both host and parasite offer several advantages. Chickens are easily reared under experimental conditions and at no great cost, while *Heterakis* is extremely useful for experimental work, being readily obtained and cultured and having a direct life history involving no intermediate host. The chicks were Light Sussex × Rhode Island Red, first cross cockerels. They were procured as day olds and reared under parasite-free conditions until they were needed for experiment. Careful faecal examinations always failed to show the presence of any parasites. They were used as young as possible as it is generally assumed that young stock are more readily parasitised than older stock.

#### DEGREE OF IMMUNITY CONFERRED BY AN EARLY INFESTATION.

Experimental evidence of such immunity was sought for twice : the procedure being the same in both cases.

In the first experiment 10 chicks were used, 5 being fed 100 infective eggs of *H. gallinae* at the age of 12 days, the others acting as controls. A fortnight later, all the chicks were fed 100 infective eggs and they were all killed 10 days later and the contents of the caeca examined.

In the second experiment the chicks were 40 days old at the time of the first feed and 53 days at the second feed.

In each case the worms from each feed could easily be distinguished at post mortem on size alone for the two groups were obvious.

In the first experiment it might seem that some immunity had been conferred on the birds but the results of the second experiment show that no such interpretation can be given to the figures, for here more worms have developed from the second feed, not only when they are compared with the controls but also with the first feed. Only one conclusion can be drawn, that in the case of *H. gallinae* the presence of worms in the caeca in no way deters the development of other worms of the species.

These results contrast strongly with those from hookworm in dogs and stomach worm in sheep, where definite evidence was obtained that an

animal can throw off a second dose of eggs or larvae more easily than the first.

Experimental Birds. (Given two feeds)				Controls. (Given second feed only.)			
		1st Feed.	2nd Feed.			No. of worms	
Chick 7.	No. of worms	29	27	Chick 12.	No. of worms	20	
" 8.	" "	9	17	" 13.	" "	10	
" 9.	" "	12	2	" 14.	" "	4	
" 10.	" "	10	1	" 15.	" "	17	
" 11.	" "	23	0	" 16.	" "	21	
Average		16.5	9.4	Average		14.4	
Chick 93.	No. of worms	0	1	Chick 85.	No. of worms	24	
" 94.	" "	36	15	" 86.	" "	38	
" 95.	" "	56	43	" 87.	" "	3	
" 96.	" "	6	17	" 88.	" "	18	
" 97.	" "	28	68	" 89.	" "	10	
" 98.	" "	4	26	" 90.	" "	49	
" 99.	" "	23	47	" 91.	" "	30	
" 100.	" "	2	12	" 92.	" "	30	
Average		19.37	28.62	Average		25.25	

#### DEGREE OF IMMUNITY WHICH DEVELOPS NATURALLY WITH AGE.

In many animals an increasing degree of immunity has been recorded as the age of the host increased. To take only one specific example, Ackert (1929) quoting unpublished results showed that unparasitised chickens developed a resistance to *Ascaridia lineata* at the age of 12 weeks. Infestations with *Heterakis gallinae* were carried out at different ages in order to investigate the state of affairs existing with this parasite.

In each case 400 eggs were administered. The chicks ranged in age from 12–209 days with intervals of about a fortnight. Post-mortem examination was carried out 24 days after infestation. The results are recorded below.

It will be seen that the birds seem fairly susceptible at all ages and there is always a good deal of variation between individual birds in a group. The greatest susceptibility seems to occur at about 10–12 weeks but even at 30 weeks a very definite infestation results. It is worth noting, however, that as the birds become older, an increasing proportion of them refuse the infestation. Yet we can only assume from these

results that very little natural immunity to *H. gallinae* develops, concurrent with increasing age.

Age of chicks in days.	No. of birds.	Total no. of worms recovered.	Range.	Average per bird.	Percentage survival.
12	5	292	9-29	66.0	16.5
16	3	56	1-18	18.4	4.6
26	5	288	4-21	57.6	14.4
44	13	650	0-113	50.0	12.5
72	8	620	0-56	57.48	19.37
85	8	808	3-49	101.0	25.25
113	6	256	0-132	22.0	5.5
126	1	42	—	—	10.05
151	3	0	—	—	—
167	9	207	0-96	23.0	5.6
186	3	108	0-101	36.0	9.0
209	6	184	0-111	30.6	7.65

The case of the three birds fed at 151 days is interesting. In all hatches it has been found that some birds exhibit a marked individual resistance to the worm. Why this should be so is unknown but as the birds were all treated alike as far as possible, there must at the moment be assumed a fundamental individual peculiarity in certain birds.

#### RELATIVE POTENCY OF A NUMBER OF SMALL DOSES OF EGGS AS COMPARED WITH ONE LARGE ONE.

In the last experiment the results of a single feed of eggs were calculated and though definite infestations resulted yet there was a heavy mortality. Under natural conditions, the birds would be unlikely to pick up even 100 eggs at the same time and it was felt that better results might be obtained if they were fed smaller doses regularly. In order to test this, two groups of three chicks were taken at the age of 12 days. The chicks of the first group were fed 10 eggs of *H. gallinae* on each of 10 consecutive days, making 100 in all. The other chicks were fed 100 eggs each on the sixth day and all were post-mortem 30 days after the first original feeding. The results were as follows :—

- Group 1. Number of worms found in chicks were 23, 5 and 22 making a total of 50, with an average of 16.6 per bird.
- Group 2. Number of worms found in chicks were 8, 1 and 5 making a total of 14, with an average of 4.6 per bird.

The numbers are too few to draw any dogmatic conclusions but it would seem that in experiments where heavy infestations are desirable, it is advisable to give several small feeds rather than one large "blunderbuss" dose. This generalisation may not of course hold good for all worms, particularly those which exhibit strong immunity reactions. In some cases, the presence of developing worms in the gut may cause later feeds to be thrown out.

In view of these results, it is interesting to notice this—that it seems impossible to build up heavy infestations of *H. gallinae* in the chicken. While it is difficult to show a definite immunity reaction on the part of the host, yet only a small percentage of eggs reach maturity in the host. Many hundreds of caeca have been obtained from local dealers and examined in the course of this work, and though considerable variation occurred, yet in very few cases could the infestation be described as "heavy." Rarely were more than 100 worms procured from a single bird and frequently only 20 or 30 could be found—often even fewer. This indicates that some kind of immunity response is occurring but it still needs to be discovered.

#### THE MOST FAVOURABLE DOSE FOR ADMINISTRATION.

In order to make the results of experiments as conclusive as possible, it is advisable to have an appreciable number of worms present. Yet to double the dose does not necessarily guarantee that double the number of worms will result in the caeca. For this reason, at the age of 12 days 7 chicks were fed 200, 300, 400, 500, 600, 800 and 1,000 eggs respectively. The chicks were autopsied after 24 days and the following results obtained.

Number of chick.	Dose.	Number of worms in caeca.	Percentage survival.
36	200	46	23·0
35	300	118	39·3
34	400	140	37·5
33	500	150	37·5
32	600	134	22·3
31	800	9	1·1
30	1,000	3	0·3

There is always a heavy mortality among the worms but the most favourable dose would seem to be as low as 300, above which a regular

falling off occurs. The results from the highest doses are very interesting as practically all the eggs are eliminated. A dose of 1,000 eggs was repeated with several chicks but the same results were always obtained. It may be that the hatching of a large number of larvae at the same time stimulates the action of the involuntary muscles so that the bulk of them are passed out and have no opportunity of entering the caeca.

While looking through the literature, a paper by Baker *et al.*, quoted in the references, was read and certain statements in it seemed to need further investigation. One statement, which incidentally has been repeated and quoted in several pamphlets of the Ministry of Agriculture and Fisheries dealing with poultry, was to the effect that the presence of *Heterakis* in the caeca increased the efficiency of the bird and encouraged its growth. If the worm was not there the caeca would not develop properly and become hypertrophied in length, with very thin walls and that they always contained a large quantity of gas. The chicks too were unthrifty and failed to grow.

This statement was investigated very simply by taking a group of young chicks, 16 days old which were all matched individually for weight. Half these birds were fed 300 *Heterakis gallinae* eggs while the controls remained uninfected throughout the experiment. All the chicks were weighed regularly. Two of the experimental birds died accidentally early in the experiment. The weights of the birds increased regularly and will not be given in detail—only the final weights.

It will be seen that so far as the final weights of the birds are concerned, there is very little difference. Each pair started out equal and the slight final differences that occur are spread between the two groups. Similarly with the lengths of the caeca—some variation occurs, even among pairs from one animal, but the longest caeca are not always found in one group. In fact the longest ones are generally in the group which was not infected. No enteritis was ever observed. Gas occurred in both groups. Only one pair of caeca was very full—that marked +++ and this is a chick which is carrying worms. No difference was noticed in the thickness of the walls. The caeca were in fact perfectly healthy. One can say emphatically then that *Heterakis* is not beneficial or in any way essential to the growth and well-being of the chick. It does not seem on the other hand to cause any damage. The host-parasite relationship has here been brought to a high degree of perfection, for the parasite never occurs in

large numbers and never attacks the gut wall at any time in its life history. It has not yet been shown to produce any lesions but there is no evidence yet that it is even useful to the host, let alone essential.

No. of bird.	Death date.	No. of worms present.	Length of caeca.		Average.	Enteritis.	Gas.	Final weight in ozs.
26	31.1.33	16	17.5	17.8	17.65	—	+	49.5
32	31.1.33	0	15.9	15.6	15.75	—	—	48.5
27	31.1.33	15	16.5	15.8	16.15	—	+++	43.0
33	31.1.33	0	17.9	19.6	18.75	—	—	47.0
29	31.1.33	22	17.1	17.0	17.05	—	+	45.5
35	31.1.33	0	19.8	20.7	20.25	—	+	46.0
25	31.1.33	90	17.0	17.0	17.00	—	+	42.5
31	31.1.33	0	16.6	17.0	16.80	—	—	45.5

Observations were made on the longevity of the eggs under natural conditions. For this purpose twelve tubes were prepared containing six to eight infective *Heterakis*. These were buried in soil outside in a garden. Once a month, one tube was exhumed, the eggs were recovered and fed to two chicks, each chick receiving two hundred infective eggs. These chicks were killed after twenty-eight days. The results are summarised in the following table. The eggs were all buried 12.x.33.

Length of time the tubes lay buried.	Date of Exhumation.	Fed to two chickens.	Chickens killed.	Number of <i>Heterakis</i> in caeca.	Percentage survival.
1 month	13.xi.32	13.xi.32	11.xii.32	26 and 24	12.59
2 "	13.xii.33	13.xii.33	10.i.33	45 " 54	24.75
3 "	13.i.33	14.1.33	11.ii.33	4 " 1	1.25
4 "	13.ii.33	13.ii.33	13.iii.33	20 " 43	15.75
5 "	13.iii.33	13.iii.33	10.iv.33	40 " 13	13.25
6 "	13.iv.33	13.iv.33	11.v.33	43 " 67	27.5
7 "	13.v.33	13.v.33	10.vi.33	19 " 13	8.0
8 "	13.vi.33	13.vi.33	11.vii.33	14 " 6	5.0
9 "	13.vii.33	13.vii.33	10.viii.33	25 " 42	16.75
10 "	12.viii.33	12.viii.33	9.ix.33	40 " 3	10.75
11 "	13.ix.33	13.ix.33	11.x.33	19 " 22	10.25
12 "	13.x.33	13.x.33	10.xi.33	22 " 16	9.5

From these results it would seem that *Heterakis* eggs can remain in the soil for a considerable time and that they are able to produce an infestation at least twelve months after reaching the infective stage. It is worth

noting that these eggs remained remarkably "clean" and showed very little growth of fungi or bacteria around them.

The work has been carried out under a grant from the Medical Research Council to Prof. R. T. Leiper, F.R.S., who also provided facilities for the actual experimentation.

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## Observations on *Paratylenchus macrophallus* (de Man, 1880).

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### INTRODUCTION.

DE MAN (1880) described the species *Tylenchus macrophallus*, obtained from pasture soil, and in a later publication (1884) gave a fuller account of it with drawings. Reasons are given later in the present paper for the removal of this species to the genus *Paratylenchus*. Micoletzky (1921) established the genus *Paratylenchus* containing but one species, namely, *P. bukowinensis*, obtained from around grass roots near Czernowitz in Bukowina. The description was based on a single female specimen which was found only after mounting in glycerine. In spite of this, however, the distinctive characters of the genus were clearly indicated and are sufficiently marked to separate it from the nearly related genus *Anguillulina*. Cobb (1923) gave a detailed description of the anatomy of another species, also obtained from around grass roots, under the name of *Paratylenchus nanus*, which he considered might be synonymous with *P. bukowinensis*, and a brief account of an immature form which he called *P. anceps* but which very closely resembled his *P. nanus*. In the case of both of these forms only females were found.

Steiner (1924) recorded the finding of more than 200 female specimens of *P. nanus* in brownish surface patches on the roots *Zinnia elegans* but again no males were found. Baylis and Daubney (1926) listed *Paratylenchus* as a synonym of *Anguillulina* with the comment that Micoletzky's account was based on a single specimen. Bally and Reydon (1931) briefly described another species under the name of *P. besoekianus* obtained from the cortex of coffee roots growing in the Dutch East Indies. In addition to females, they found males which were much rarer than females. Their

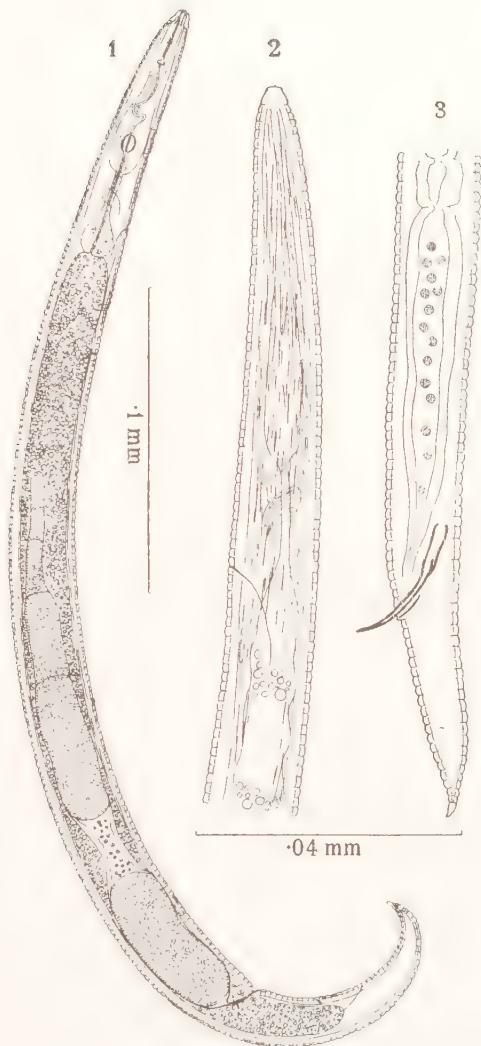
description and drawings show that *P. besoekianus* is very closely related to if not identical with the forms already described under the names of *P. bukowinensis*, *P. nanus* and *P. anceps*; this question is, however, discussed later on in the section dealing with systematics.

In investigating nematodes associated with the roots of grasses, the writer has found a species of *Paratylenchus* constantly and in good numbers in extracts, made by the Baermann funnel method, from pasture soil at Winches Farm. Male specimens have been discovered, though in far fewer numbers than females and on only two or three occasions. A careful examination of the adults of both sexes has revealed certain very interesting structural features, whilst other observations point to the semi-parasitic habit of the worms. In the present paper an account is given of these observations and the systematic position of the worms is discussed.

#### MORPHOLOGY.

Dimensions :—*female* : length, 0·311 mm. to 0·497 mm.; stylet, 15 $\mu$  to 56 $\mu$  long, eggs, 50-60 $\mu$  long by 18-20 $\mu$  wide;  $\alpha=24\cdot4-16$ ,  $\beta=7\cdot44-4\cdot66$ ,  $\gamma=12-10\cdot5$ ,  $V=78\cdot7\%-85\cdot4\%$ ; *male* : length, 0·32 mm. to 0·42 mm., spicules, 22-25 $\mu$ , gubernaculum, 4-5 $\mu$ ,  $\alpha=23\cdot5-21$ ,  $\beta=4-3\cdot5$ ,  $\gamma=13-11\cdot4$ .

There is considerable structural difference between the adult females and males; the following account is, therefore, based on the anatomy of the female. Small worms; the largest found being less than 0·5 mm. in length. Body tapering a little anteriorly and considerably posteriorly from the vulva to the tip of the tail, which is often produced into a small process. As found in water extracts from turf, the worms mostly lie more or less bent towards the ventral surface. They move rather slowly. Cuticle with rather coarse transverse striae. Head shaped like a truncate cone and separated from body by a very slight constriction. Under high magnification, surface of head shows 6 faint ridges which possibly indicate the outlines of the original lips. Buccal cavity a simple straight sided tube about as long as depth of head. In some specimens it seems to be continued behind the level of the head for a short distance, curving outwards on either side, as figured by Cobb for *P. nanus*. In most specimens, however, this region is very indefinite. Stylet well developed and remarkably variable in length; in some cases only 15 $\mu$  long, in



*Paratylenchus macrophallus* (de Man).

Fig. 1.—Adult female under low magnification to show general shape and structure ; lateral view.

Fig. 2.—Head and oesophageal region of male ; highly magnified.

Fig. 3.—Posterior part of male, in lateral view ; highly magnified.

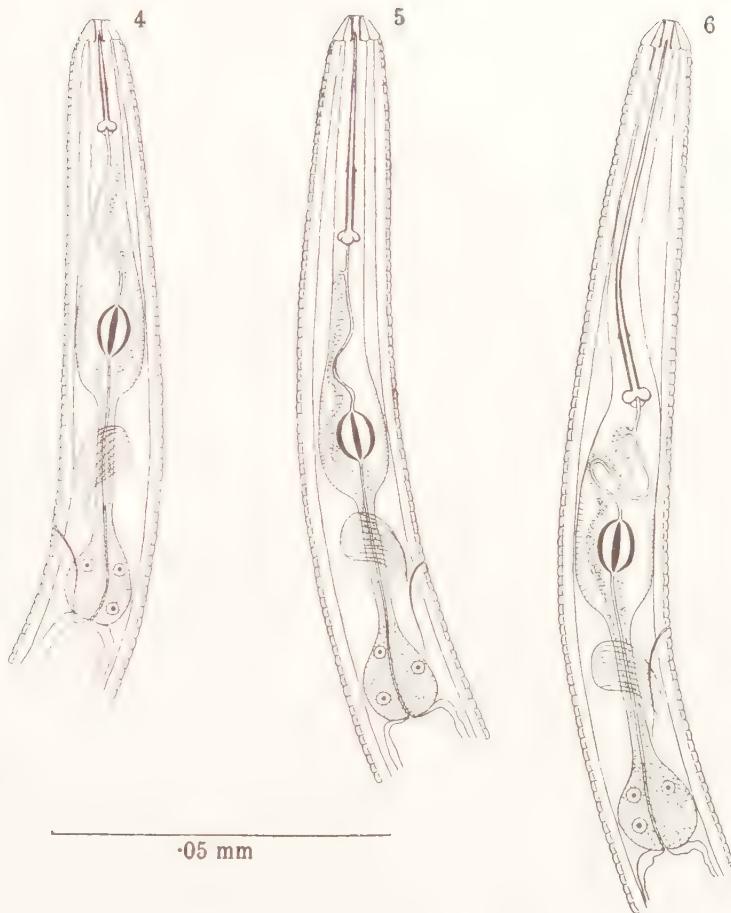
others as much as  $56\mu$  long, as shown in figs. 4–6. It has the same structure as in species of *Anguillulina* and consists of an anterior portion which is conical and tapers towards the point, and a posterior cylindrical portion which carries three basal swellings. The junction of the two portions is marked by a small projection on either side formed by the overlapping of the anterior region. It is noteworthy that whatever the total length of the stylet, whether comparatively short or remarkably long, the length of the posterior part remains fairly constant whilst the anterior part varies considerably in length. Another interesting fact is that the length of the stylet is not correlated with the total length of the body. Short specimens may have short or long stylets and the same is true of longer specimens as the following examples show:—

Total length.				Length of Stylet.
0·311 mm.	...	...	...	15 $\mu$
0·335 mm.	...	...	...	50 $\mu$
0·44 mm.	...	...	...	17 $\mu$
0·497 mm.	...	...	...	56 $\mu$

On the whole, forms with very short or very long stylets are not so plentiful in a given extract as those with stylets of about  $27\mu$  or  $30\mu$  long, but specimens having short, medium and long stylets have almost always been found side by side in extracts from one and the same piece of turf. In fact the great variability in the length of the stylet is one of the most remarkable anatomical features of this organism.

Correlated with the length of the stylet is the length of the pre-bulbar portion of the oesophagus as shown in figs. 4–6. This region is not a narrow portion distinct from the muscular oesophageal bulb as in species of *Anguillulina* but is continued backwards from the base of the stylet and gradually expands into a rather elongate club-shaped bulb. The lumen of the stylet is continued backwards into that of the oesophagus as a clear narrow tube following a more or less twisted course. When the stylet is short the lumen has a rather wavy course. When the stylet is of medium length the course of the lumen is still more bent, whilst in the case of long stylets the lumen is much coiled and twisted. At the centre of the bulb are three comparatively large crescentic thickenings behind which the lumen is continued as a narrow tube.

Following the median bulb, there is a narrow neck, having about the same length in all forms, which finally expands into a rather rounded or



*Paratylenchus macrophallus* (de Man).

Figs. 4, 5 and 6.—Head and oesophageal region in three different worms showing the great variations in the length of the stylet.

flask-shaped terminal oesophageal bulb. This is glandular in structure, not muscular as suggested by Micoletzky, and contains the three oesophageal glands, the nuclei of which can often be distinguished in freshly killed specimens on examining under the oil-immersion.

As in species of *Anguillulina*, the dorsal oesophageal gland empties into the lumen of the oesophagus by a duct a short distance behind the base of the stylet. The sub-ventral glands also open as in species of *Anguillulina* just where the crescentic thickenings join the lumen leading to the neck of the oesophagus. The nerve ring crosses the neck of the oesophagus and the excretory pore varies in position relatively to the posterior oesophageal bulb, according as the stylet and the fore part of the oesophagus are short or long. In the former case it lies, as in fig. 4, close to the posterior bulb, in the latter case, as in fig. 6, it is placed well forward.

Intestine well stocked with fatty food globules, terminating in a rather obscure rectum which empties at the anus, often quite inconspicuous. Vulva in form of a broad ventral slit and having a rather prominent rounded anterior lip. Vagina short, leading to uterus with stout walls and capable of holding one egg at a time. Numbers of small rounded spermatozoa frequently seen in uterus. Ovary single, extending anteriorly in body and gradually tapering to front end which is not reflexed on itself. There is no post-vulval uterine sac such as is indicated in the drawings of Micoletzky and Bally and Reydon.

*Male.* As has already been pointed out, adults of this sex are of much less frequent occurrence than females, in fact, they may be described as rare. The writer has obtained them in water extracts from turf on only three occasions and then only in small numbers. Body on the whole smaller and narrower than in female; tapering a little anteriorly and considerably posteriorly to form a sharply pointed tail. Cuticle transversely striated. Head shaped as in female but surface ridges very indistinct or absent. Mouth aperture absent. Stylet absent or so inconspicuous as to be merely shadowy in outline. Oesophagus very indistinct, the merest outline being visible under high magnification. The bulbous region indicated in fig. 2 is shown more clearly than it actually appeared in the specimen itself. A neck region apparently present and crossed by the nerve ring. Terminal part of oesophagus very indefinite, no distinct glandular bulb discernible. Intestine rather vacuolate in appearance with short transverse bands of

fat globules crossing it here and there. Spicules very slender, sharply pointed. Anterior third expanded to form a rather elongate head region with the dorsal and ventral edges curving inwards. Gubernaculum very short and simple. Caudal alae absent. Gonad single and extending forwards to a point a little in advance of the middle of the body, front end not reflexed. Posterior part forming a vas deferens with fairly stout walls. Small round spermatozoa recognisable in this part. Although lacking a well formed stylet and oesophagus when adult, the males appear to be sexually functional since they possess comparatively large spicules and spermatozoa occur in the vas deferens.

Cobb observed spermatozoa in the uterus of female worms and this, coupled with the absence of males, led him to express the opinion that the females are probably syngonic, *i.e.*, the gonad is capable of producing both spermatozoa and ova. This possibility is not, of course, excluded by the finding of apparently functional males since, so far as present observations go, these are so few in numbers that there would seem to be insufficient of them to inseminate the numerous females which are found in extracts from turf.

#### BIONOMICS.

de Man found *Tylenchus macrophallus* in pasture soil. Micoletzky obtained his single specimen of *P. bukowinensis* from soil around grass roots. Cobb's *P. nanus* also came from around grass roots and his *P. anceps* from the roots of *Umbellularia californica* Nutt., a timber tree known as the Californian Olive. Steiner's specimens of *P. nanus* were present in surface lesions of the roots of *Zinnia elegans*, an annual bedding plant, whilst *P. besoekianus* of Bally and Reydon was obtained from brown spots in the cortex of coffee roots. Although, up to the present time, the writer has not found specimens within grass roots, his observations show that the worms live in intimate association with such roots. In making extracts from turf by the funnel method, worms were much more abundant from unwashed than from washed roots, which would seem to suggest that they are mainly free in the soil. Nevertheless, the fact that they were obtained from roots from which the soil had been washed away goes to show that they were living in close association with the roots and were possibly entangled in the root hairs. This last site would seem, in fact, to be their habitat since on examining some grass roots which had been fixed in

Flemming's solution and afterwards cleared and mounted in Canada balsam, a specimen of the worm was found with the head abutting on the root, into which the stylet was inserted, whilst the body was surrounded by root hairs. These observations, coupled with the earlier records made by Steiner and Bally and Reydon of their occurrence in the outer layers of roots go to show that the worms are probably to be regarded as semi-parasites.

#### SYSTEMATICS.

Up to the present time the following species of *Paratylenchus* have been described : *P. bukowinensis* Micoletzky, 1921, *P. nanus* Cobb, 1923, *P. anceps* Cobb, 1923 and *P. besoekianus* Bally and Reydon, 1931. When we consider the status of these it is found that they differ from one another in such minute details as to render it probable that they all belong to one and the same species.

Cobb said that *P. nanus* may be synonymous with *P. bukowinensis* and it would certainly seem from his description and drawing that this is so. *P. anceps* was based on immature material but there were indications that the vulva would occupy the same relative position as in *P. nanus*. The stylet was longer than in *P. nanus* and the striations of the cuticle were only 1 $\mu$  apart as compared with 2 $\mu$  apart in *P. nanus*. In view of the remarkable variations in the length of the stylet observed by the writer, it is clear that the separation of forms into distinct species, according as they possess a short or long stylet, cannot be considered a valid procedure. The narrower cuticular striations of *P. anceps* may have been due to its immature condition and can scarcely be considered as of sufficient importance as to establish this as a distinct species.

*P. besoekianus* appears to be smaller than *P. bukowinensis*; the average length of 6 females is given by Bally and Reydon as 0.254 mm. and of 2 males as 0.22 mm. The drawings of the female show that they are closely similar in appearance and structure to those found by the writer. The shape of the body is practically identical and it is concave on the ventral surface. The vulva is in the same relative position and the oesophagus has almost the same shape. The male also has the same general appearance as those found by the writer; the stylet and oesophagus being merely shadowy, whilst the tail has the same shape. The spicules have a rather stouter appearance than those figured by the writer but the gonad is

comparatively short, as in the specimens obtained from turf at this Institute. Bally and Reydon state that the stylet of the female is shorter than that of *P. bukowinensis* but, as has been pointed out above, this organ is so variable in length that it cannot be used as a means of separating species. Altogether it seems reasonable to suggest that all these forms belong to one and the same species.

In discussing *P. nanus*, Cobb drew attention to its close resemblance to *Tylenchus macrophallus* de Man and suggested that if opportunity arose it might be advisable to re-examine the median oesophageal region of *T. macrophallus*. de Man's drawing of this with the bend in the lumen is certainly very similar to the appearance presented by this region in the forms figured by Micoletzky, Cobb and the writer in the present paper. The size, general shape of the body with truncate head and tapering tail, position of the vulva and the long stylet all reveal the remarkable similarity of the worms described by de Man to those forms since placed in the genus *Paratylenchus*. de Man himself drew attention to the fact that in *T. macrophallus* the stylet and oesophagus are more strongly developed in the female than in the male; a further point of agreement with what has been written above concerning *Paratylenchus*.

Cobb concluded that "for the present at least it seems best, unless the undiscovered male of *nanus* should prove to be extraordinarily like the male of *macrophallus*, to regard the two species as distinct." Now the tails of the males observed by the writer are extraordinarily like that figured by de Man for *T. macrophallus* especially in the shape of the long slender spicules and the short gubernaculum. The only point of difference is that very short and narrow caudal alae are shown as present on *T. macrophallus* whereas they are absent from males of *Paratylenchus*. One hesitates to suggest that so accurate an observer as de Man may possibly have been in error with regard to the presence of caudal alae, but if we admit the possibility the way is clear for the removal of *T. macrophallus* to the genus *Paratylenchus* which the writer accordingly does, herewith.

Reasons have already been adduced for regarding all the species of *Paratylenchus* so far described as being one and the same and it is now clear that they are to be considered as identical with the worms described by de Man under the name of *Tylenchus macrophallus*. Since, however, this species was made in 1880 its specific name has priority and the worms must in consequence be named *Paratylenchus macrophallus* (de Man, 1880);

the following names being synonyms :—*P. bukowinensis* Micoletzky, 1921, *P. nanus* Cobb, 1923, *P. anceps* Cobb, 1923 and *P. besoekianus* Bally and Reydon, 1931.

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## On *Syngamus ierei* sp. nov. from Domestic Cats, with some Observations on its Life-cycle.

By J. J. C. BUCKLEY, M.Sc.

(Wandsworth Research Scholar, London School of Hygiene and Tropical Medicine.)

THE species to be described was found in a high percentage of domestic cats from widely distributed localities in Trinidad, and the material was collected by the writer during a recent visit to the British West Indies. It was found to be present in cats in various parts of the island, including Port of Spain, St. Augustine, Manzanilla and Mayaro. Of thirty-two cats examined, fifteen, or nearly 50 per cent. harboured the parasite, in numbers varying from a single pair to a maximum of twenty-seven pairs. In habitat, they were restricted to the nares; on one occasion a pair was found in the stomach, but this probably must be regarded as an accidental habitat, as the worms were unattached to the mucosa, and had doubtless been swallowed after becoming detached from the nasal mucosa. The trachea was never found infested.

### MORPHOLOGY.

*Female*.—The body tapers gradually from the vulvar region to either extremity. At the anus it narrows rather abruptly and forms a tapering tail, from the tip of which a pair of lateral papillae are situated a short distance. The average length of seventeen mature worms was 20·3 mm., the maximum length being 23·8 mm. The distance of the vulva from the anterior end of the body averages  $\frac{1}{3}\cdot\frac{1}{2}$  of the total body length, and varies from  $\frac{2}{3}\cdot\frac{1}{9}$  to  $\frac{3}{3}\cdot\frac{1}{8}$ . The distribution of the ovarian tubules and uteri is somewhat variable, and is illustrated in the diagram (Fig. 8). It will be seen from this that the uteri range on either side of the vulva to a distance which is always less than half the length of the worm and that the ovaries do not extend posteriorly as far as the anus, or anteriorly to the oesophagus. The oesophagus (including the buccal capsule) measures 1·1 to 1·4 mm. in length and its ratio to the body length varies

from 1 : 10·8 to 1 : 17·5. The excretory pore, with which are associated a large pair of cervical glands, is situated about the level of the posterior end of the oesophagus. The cervical papillae are at the same level, and are non-protuberant. The buccal capsule is broader than long, measuring 0·4 to 0·55 mm. broad, by 0·25 to 0·4 mm. long. In lateral view it is somewhat asymmetrical, as the junction with the oesophagus is displaced slightly to the ventral side of the worm. In end-on view the capsule is seen to be thrown into folds which represent the six indentations described in *S. laryngeus* and *S. trachea*, but this character is not very well marked and in some cases it is almost completely obliterated (Fig. 4). The six oral papillae are difficult to see but are situated in the usual position relative to the axis of the body. At the base of the capsule are eight prominent and stoutly-built teeth, but no supporting ribs extend up the sides of the capsule as have been described in other species of the genus.

*Male*.—The length varies from 5 to 6·9 mm. and the body is about equal in breadth over the whole of its length. The ratio of the oesophagus to body varies from 1 : 5·3 to 1 : 6·6. The buccal capsule is broader than long, about 0·3 × 0·28 mm., and its walls are relatively thicker than in the female and its shape is more cylindrical. Other characters of the buccal capsule are similar to those described in the female. The bursa is of the usual fleshy stumpy type with short thick rays whose shape and relative dimensions are seen in Fig. 5. The spicules are easily discernible on clearing the specimens in beechwood creosote. They are sub-equal, the right spicule usually being a little longer than the left. Four pairs were examined and were found to be respectively 36 and 44, 44 and 48, 44 and 58, and 47 and 52 $\mu$  in length.

#### RELATIONSHIPS.

The species under discussion has affinities with *S. felis* Cameron, 1931, whose host is also feline, viz. : the Malay Tiger (*Felis tigris*). Characters of resemblance between these two species are :—

1. The relatively long tail in the female.
2. The presence of a pair of papillae some distance from the tip of the tail in the female.
3. The somewhat cylindrical shape of the male buccal capsule.
4. The non-protuberant cervical papillae.

*Syngamus ierei* sp. nov.

- Fig. 1. Anterior end of female. Lateral view.
- .. 2. " " " male. " "
- .. 3. " " " female. Dorso-ventral view.
- .. 4. End-on view of buccal capsule. (V=ventral. D=dorsal).
- .. 5. Bursa.
- .. 6. Tail of female. Lateral view.
- .. 7. Spicules.

*Syngamus ierei* differs from *S. felis* in the following characters:—

1. There are no supporting ribs to the mouth capsule.
2. The general dimensions and dimension of the mouth capsule are smaller.
3. The spicules are larger, e.g., 36 to 52 $\mu$  compared to 25 to 30 $\mu$  as in *S. felis*.

The remaining mammalian species of *Syngamus* can be distinguished from the present species by means of one important character, viz.: the presence of ribs in the buccal capsule. These structures are present in varying numbers in *S. kingi* Leiper, 1913, *S. laryngeus* Railliet, 1899, *S. nasicola* Linstow, 1899, *S. hippopotami* Gedoelst, 1924 and *S. indicus* Monnig, 1932. The description of *S. dispar* Diesing 1851, is inadequate, but in Diesing's (1857) figures of this species, buccal ribs appear to be drawn, and on these grounds and the difference of host the present species is considered distinct from it. The question of its possible identity with *S. kingi* has been given special attention, for in his account of this species Leiper suggested that its normal host might prove to be cat or dog. The differences in structure between these two species, however, are such as to leave no doubt as to their separate identity. Not only are buccal ribs present in *S. kingi*, but it also differs in the short stumpy tail of the female, the presence of protuberant cervical papillae and in the apparent absence of spicules. Further the writer has in another publication (1933) discussed the probable identity of *S. kingi* with *S. nasicola* Linstow which has recently been found occurring commonly in ruminants in the West Indies.

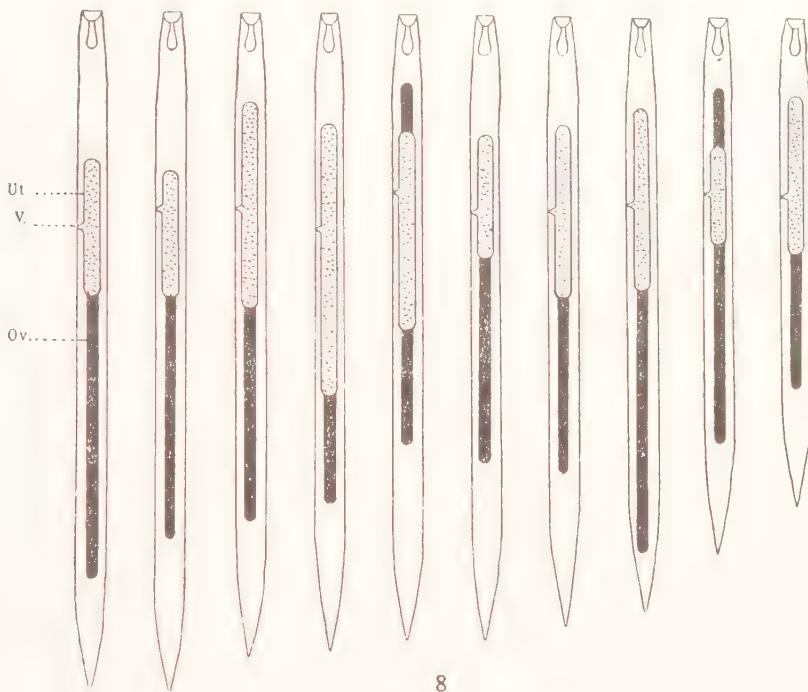
The above points appear to be sufficient for regarding the present species as new, and the name *S. ierei* is given to it.

With regard to the known occurrence of *Syngamus* in mammalian hosts, it may be mentioned that De Does (1907) stated that he found *Syngamus* in horses and dogs, but no description of these is given.

#### LARVAL DEVELOPMENT: EGG TO 3RD STAGE LARVA.

The eggs average 92  $\times$  49.5 $\mu$ , the maximum length being 100 $\mu$  and minimum 84 $\mu$ , and the maximum breadth 52 and minimum 48 $\mu$ . The shell is marked by fine irregular transverse striations which give it a pitted appearance in optical section. Polar caps are absent, and

in this character the species shows a striking difference from *S. trachea* and conforms with what is probably a general characteristic of mammalian Syngamids. When passed in faeces the eggs are typically in the 4 to 6-cell stage.



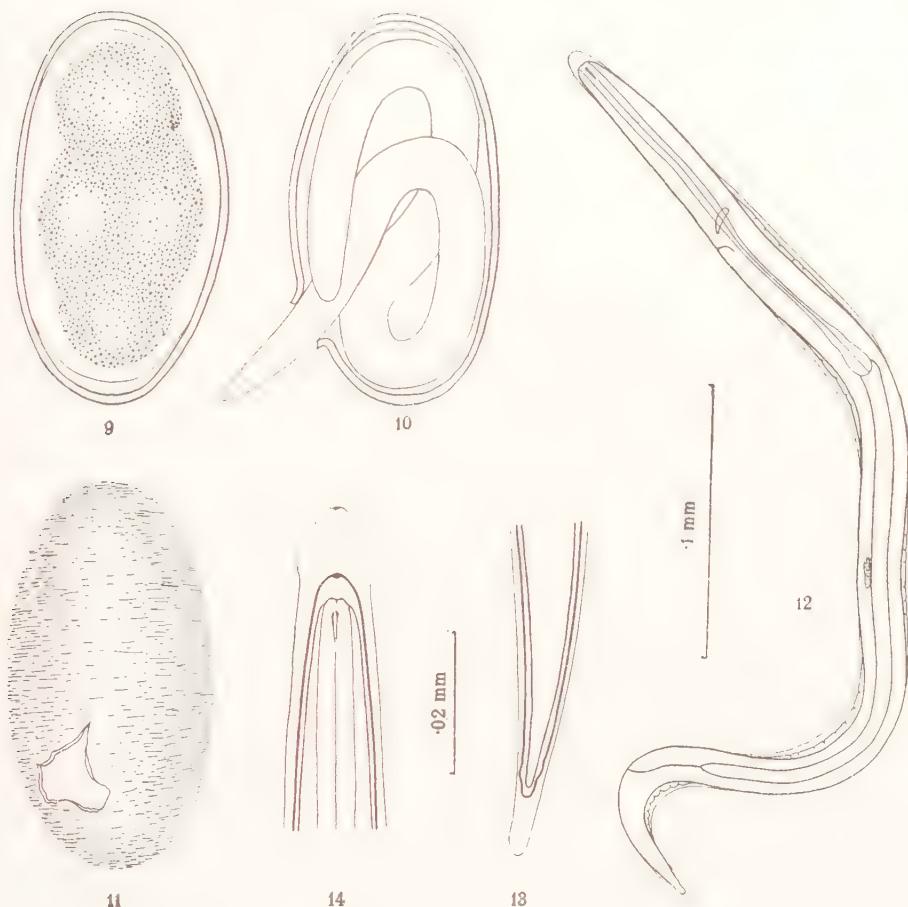
*Syngamus ierei* sp. nov.

Fig. 8. Diagram of disposition of female genital organs.

At a temperature of 26–30°C., segmentation of the egg contents proceeds rapidly and after 3 to 4 days a larva is formed (1st stage) which moves sluggishly inside the shell. It measures 0·32 to 0·4 mm. long and little of its organisation is discernible owing to the closely packed granules which occupy nearly the whole of the body with the exception of the anterior and posterior extremities.

The second stage is attained after 5–6 days, and larvae hatched artificially, by pressure, showed a delicate sheath a little beyond the tail and less beyond the head. A mouth capsule is represented by a slight thickening of the cuticular lumen of the oesophagus. Owing to the presence of numerous granules in this region the oesophagus could not be seen. The genital rudiment however is visible, as are also the anus and excretory pore. The second stage larva measures about 0·45 mm. long.

After eight days the third stage larva has completed its development and hatching begins. This takes place by an irregular jagged rupture of the shell (Fig. 10) which is nearly always subterminal in position. In this respect it differs much from *S. trachea* in which, according to Ortlepp (1923), hatching takes place by the embryo pushing its way through one of the openings in the shell caused by displacement of the polar cap. The hatched larva is provided with two sheaths, a very delicate outer sheath which has been retained by the larva after the ecdysis which followed the metamorphosis from the first to the second stage. This sheath projects beyond the larva at each end. Anteriorly there is a small internal knob (Fig. 14) indicating the remains of the mouth capsule of the 1st stage larva. The sheath is slightly expanded annularly a short distance from the end. The inner sheath is much stouter, resembling that of a hookworm larva, but does not project at each end. It is the same length as the larva itself and is very closely applied to the body both at the head and at the tail. By means of pressure under a coverglass the sheath can be separated from the body at each end, and anteriorly it is seen to have an internal knob as in the outer sheath. These two sheaths imply two metamorphoses, therefore the larva is in the third stage. It is about 0·45 mm. long by 0·016 mm. broad. (Fig. 12.) The mouth-opening is minute and subjacent to it are a pair of indistinct buccal spears. Sub-terminal cuticular depressions suggest the presence of papillae. The oesophagus is usually more than  $\frac{1}{3}$  the body length, and has a posterior bulb joined by a slender part to an anterior cigar-shaped portion, around the latter part of which is the nerve ring. The excretory pore is about the same level. The intestine and surrounding body space is packed with granules. The anus is about 0·05 mm. from the tip of the tail. The genital rudiment is situated about the middle of the body.

*Syngamus ierei* sp. nov.

- Fig. 9. Egg as passed in faeces.
- „ 10. Emergence of larva from egg.
- „ 11. Egg showing transverse striations and aperture in shell caused by emerging larva.
- „ 12. Third stage larvae.
- „ 13. Tail of 3rd stage larva.
- „ 14. Anterior end of 3rd stage. (Note the two sheaths. The inner sheath has been forced away from the larva by pressure under a cover glass.)

In the act of emerging from the egg, the larva often exsheathes from its outer sheath. If, however, it is still retained, it is generally lost a day or two after hatching, although it has been seen once on a larva hatched for twelve days. Exsheathment from the inner sheath occurs spontaneously after about twelve days. By this time the granules in the body have mostly disappeared and are seen only in the intestine.

The biology of the larva is similar to that described by Ortlepp for *S. trachea*, i.e., it is not negatively geotropic; it shows no thermotropism but increases in activity when the temperature of the surrounding medium is raised; it is non-resistant to dessication, and when placed on human skin no evidence of penetration was experienced.

#### ATTEMPTED EXPERIMENTAL INFECTION OF CATS.

In this experiment four cats and four kittens from Mayaro were used. The eggs for the larval culture were obtained by teasing out the uteri of adult worms taken from the pharynx and nasal passages of an infected cat, and were cultured in about 1 mm. depth of tap water in a petri dish. The culture was begun on 13.9.32, and the spontaneously hatched larvae were fed to two cats and two kittens. On 27.9.32, two hundred larvae were fed, by squirting them in water into the back of the throat with a pipette, to each of two cats and one kitten, and a thousand larvae to one kitten. Two cats and two kittens were used as controls. Two days later one of the cats was given a further five hundred larvae, and on 5.10.32 the same cat was given two thousand more larvae. Faeces of the eight animals was examined daily from 6.10.32 until 15.11.32. Fourteen days after the beginning of the experiment one of the control cats was found to be passing *Syngamus* eggs. No *Syngamus* eggs were found in the faeces of any of the other animals during the period 27.9.32 to 15.11.32 after which the experiment was closed.

It would appear from this that the direct mode of infection does not take place in this species of *Syngamus*. Although the direct life cycle obtains in *S. trachea*, it has been known for some time that earthworms can act as vectors of the infective larvae which attain and infect the definite host on the ingestion by the latter of the earthworm vectors. Further it has been shown (Clapham 1934) that a higher percentage of larvae attain maturity in the definitive host by this indirect method

of infection than if they had been fed directly to the first host. The possibility suggests itself that in the case of *S. ierei* the indirect method is the *only* mode of infection and that a vector, which is alternative but optimum for the life cycle of *S. trachea*, has become indispensable in the life cycle of *S. ierei*. Experiments are at present in progress to test this hypothesis.

#### ACKNOWLEDGMENT.

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#### SUMMARY.

1. A new species of *Syngamus* from the pharynx and nares of domestic cats in Trinidad is described.
2. The larval development from egg to 3rd stage larva is described with particular reference to the anatomy of the 3rd stage larva.
3. An attempt to trace the life cycle of the worm by direct infection of cats with the 3rd stage larvae was unsuccessful. It is suggested that an intermediate host is essential in the life cycle of this species.

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## On the Development, in *Culicoides furens* Poey, of *Filaria* (= *Mansonella*) *ozzardi* Manson, 1897.

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THE present paper is an amplification of a preliminary note by the writer (1933) in which the successful experimental infection of sand-flies with larvae of *Filaria ozzardi* was briefly described. Therein also a brief account of the known geographical distribution of this parasite was given, and previous researches on its life-cycle were summarised. It remains therefore to describe more in detail the larval development in the sandfly and the methods employed in the experimental work on which these observations were based.

The first investigations into the life-cycle of *F. ozzardi* were carried out by Low (1902) in St. Lucia and in British Guiana. Using mosquitos reared from larvae he found by direct infection experiments in St. Lucia that *Culex fatigans*, *Aedes aegypti* and *Anopheles albipes* were inefficient as intermediate hosts, and while in British Guiana he experimented with other species of mosquitos, but without success. He also examined fleas (*Pulex irritans*) and chiggers (*Pulex penetrans*) from infected cases in this region but found no larvae in them. Fülleborn (1908) fed eleven *Anopheles maculipennis* on the blood of an infected case from the West Indies, but from only three of these were larvae recovered, and although thirteen days after the infective feed, the larvae were still apparently in the "sausage" form. Of nine *Aedes aegypti* fed on the same case, only one was subsequently found infected, and entirely negative results were obtained with six *Ornithodoros moubata*. In more recent times the chief research on this problem seems to be by Vevers (1924) in British Guiana and Davis (1928) in Northern Argentina. Vevers obtained evidence which suggested that *F. ozzardi* infection amongst the Arawaks was confined to families, and he argued from this that the vector was likely to be some insect closely associated with the benab. He found bed-bugs in the hammocks of an infected family but found none in the case of uninfected families. No larvae, however, were found by him in twenty bed-bugs which were sectioned. Davis fed bed-bugs (*Cimex lectularius*)

and Triatomas (*Triatoma infestans*) on the blood of a case infected with a microfilaria which has been regarded as a new species, *F. tucumana* (Biglieri and Araoz, 1917) for some time, but has been shown (Vogel, 1927) to be identical with *F. ozzardi*. The results of Davis's experiments with these insects were negative, for on subsequent examination of the insects, up to eighteen days after being fed, microfilariae were only found in the gut and had undergone no development. Using six species of mosquitos, *Aedes aegypti*, *Anopheles albitalis*, *A. tarsimaculatus*, *A. pseudopsunctipennis*, *A. argyritarsis* and *Culex quinquefasciatus*, he found developing larvae in a small percentage of the first three species. The larvae were only found in the thorax and although of twelve and seventeen days growth were not longer than 300 $\mu$ . The head was not found infected.

The writer's experiments were carried out during the early summer of 1933 in St. Vincent in the course of a visit to the West Indies on helminthological research. St. Vincent was chosen for the work on the advice of Professor R. T. Leiper, for Low (1902) had shown that *F. ozzardi* was common amongst the people in certain parts of the island, and Cameron observed that the infection was still endemic there in 1929. An adequate supply of experimental material was thus assured together with the probability that some common blood-sucking insect in an endemic locality would be found to be acting as intermediate host for the parasite. Headquarters were made at Kingstown, the capital town, and laboratory facilities were kindly provided at the Colonial Hospital by Dr. Stanley Branch, Chief Medical Officer at St. Vincent, for whose kindness and co-operation throughout the work the writer wishes to record his grateful thanks.

#### SOURCE OF MATERIAL.

A source of infective material for the experiments was at first sought amongst the patients at the hospital and the inmates of the poorhouse at Fort Charlotte. Of thirty patients, resident in various parts of the island, whose blood was examined, only one—an old man aged 71 from Calliaqua—was found infected with *F. ozzardi*. Of twenty-one of the poorhouse inmates, two old women from Georgetown and Calliaqua respectively were infected, together with a young native of twenty from Bequia, one of the Grenadine islands near to St. Vincent. It was significant that of the four infected cases two were from Calliaqua, a small country town about four miles east of Kingstown on the leeward coast,

for it was at this spot that Low had recorded a 26.6% infection in 1902. The writer was further assured by Dr. Branch that plenty of infected cases could be found there. In the search for a possible intermediate host, it seemed advisable therefore to consider the blood sucking insects from this locality in particular and thus reduce the possibility of selecting a non-vector for the experimental infections, since the actual vector might have a limited distribution within the island, as had been suggested by Low with reference to the restricted distribution of the parasite. According to local information sand-flies, later identified as *Culicoides*, were the commonest and most annoying blood-sucking insects in Calliaqua, and on this evidence, together with the fact that *Culicoides* had been found by Sharp (1927) to be the vector of *F. perstans*, it was decided to use sand-flies in the experimental work. Furthermore, *Culicoides* was hitherto an untried vector in this life cycle. The mosquitoes *Culex infictus* Theo. and *Deinocerites cancer* Theo. were also collected in Calliaqua, but were not employed in any of the experiments.

#### PRELIMINARY EXPERIMENTS WITH *CULICOIDES*.

Numbers of sand-flies were collected at Calliaqua in test tubes plugged with cotton wool. The method was simply to wait in a room from sunset onwards with arms and legs bared and to tube the flies as they alighted on the skin, being located easily in spite of their small size, by the irritation of the bite. The flies were brought to the hospital where it was hoped to feed them on the blood of the infected case from Calliaqua. Several prolonged attempts both at night and during the day were made to induce the flies to feed by removing the cotton wool plug and inverting the test-tube containing from one to a dozen flies on the wrist or forearm, but without success ; and although ultimately no doubt some of them would have fed, the time expended and the obvious impatience of the subject made this method impracticable and it had to be abandoned.

Having noticed, however, the ease with which sand-flies would feed after alighting naturally on the skin when collecting them for the above experiment, it was thought that if the collecting could be done by an infected person, who would allow the fly to take a full meal of blood before tubing it, then the two operations of collecting and infecting would be combined in one and the above difficulties would be overcome. To find a suitable subject for this purpose a survey of the *F. ozzardi* incidence of the

inhabitants of Calliaqua was made. Blood smears were taken from sixty-seven persons and in twenty-four of these the typical larvae were found. Thus, together with the two positive cases from Calliaqua at Kingstown, the percentage of infection was 37.7%. In addition to choosing in particular, likely subjects to be used for the experiments, as a matter of interest others young and old of both sexes were examined, and the results are here summarised :—

Inhabitants of Calliaqua examined for <i>F. ozzardi</i> .		
No. examined.	No. infected.	Per cent. infected.
69	26	37.7
41♂	13	31.7
28♀	13	46.4

Only the larvae of *F. ozzardi* were found and the blood of the two subjects chosen for the experiments was examined at night for *F. bancrofti* but this infection was not found. The subjects, natives aged 25 and 28 respectively, received full instructions as to the method of collecting and feeding and were provided with test tubes and cotton wool plugs for the purpose. Supervision of this operation was only carried out at first, but examination of the flies with a hand lens sufficed to tell whether they had been given a full feed of blood, and this was further ensured by only paying the collectors for well fed flies. The flies, collected and fed from sunset onwards at Calliaqua, were brought to the laboratory the following morning in batches varying from about 3 to 35. The numbers of flies collected depended to some extent on weather conditions, for on other than still almost windless evenings very few could be obtained.

During the period 27.iv.33 to 15.v.33. batches of fed flies were received on fourteen days and numbered 191. Various methods and conditions were used in endeavouring to keep the flies alive in order to observe the fate of the microfilariae ingested in the blood meal. They were kept in ones or twos in test-tubes either plugged with cotton wool or closed with a piece of muslin ; or in a 12 in. by 12 in. by 12 in. muslin covered cage with a sleeve on one side ; or in a large bell-jar with muslin tied over its mouth. Different conditions of humidity were attempted by means of wet cotton wool in the cage or tubes, and different degrees of light and darkness were also provided. As to feeding, the opportunity of a blood meal was given almost daily but was not availed of by the majority of the flies. They were often seen, however, sucking the juices from the inner

surface of pieces of banana skin put inside the receptacle. None of these methods of keeping the flies proved successful and from the table of results it is seen that the highest mortality occurred the third day after the infective feed, and only two flies survived until the eighth day.

TABLE I.

Preliminary experimental infection of *Culicoides* with *F. ozzardi* larvae.

Days.	1	2	3	4	5	6	7	8	
Number of flies dying each day after infective feed ... ...	16	59	64	22	4	6	0	2	Total number of flies = 173
Number of flies examined ... ...	6	35	39	11	2	5	0	2	Total examined = 100
Number of flies infected with larvae in thorax	1	7	6	1	0	1	0	0	Total found infected = 16

The flies were dissected according as they died, and 16% of them were found to be infected with larvae in the thorax. In fourteen of these, which died two or four days after the infective feed, the larvae, varying in number from one to five, were in the typical "sausage" stage, while in two flies the larvae were in a more advanced stage of development. One of these flies was in the sixth day of infection, and the 3 larvae, measuring about 0.7 mm. in length were evidently the result of the experimental infection. The other fly was dissected only three days after the infective feed and the single larva found in the thorax was undoubtedly the result of a natural infection, and as it was similar in structure to the larvae in the other fly it is assumed that it was *F. ozzardi*.

These observations which were in the nature of preliminary studies in order to determine the line of work, were temporarily relinquished about the middle of May, 1933, as a visit had to be made to St. Kitts before the onset of the rainy season. It was apparent from the results obtained so far, that *Culicoides* would probably prove to be the vector of *F. ozzardi*, for the six-day larvae found on the one occasion were far in advance of the "sausage" stage, beyond which no larvae had been found to proceed in other experimental vectors by previous workers. The rather small figure of 16%, however, which represented the percentage of flies which ingested microfilariae along with the blood meal, was not very encouraging,

and it was also evident that improved methods of keeping the flies alive would have to be used if the development of the larvae was to be studied completely. The presence of at least one naturally infected fly in the series indicated that a census of naturally infected flies would have to be taken for comparison with the experimental infections.

#### FURTHER EXPERIMENTS WITH *CULICOIDES*.

On returning to St. Vincent towards the end of June 1933, two cages were made which were thought to simulate as nearly as possible the conditions and environment which Sharp (1927) described in his successful work on the life cycle of *F. persians*. The cages were 8 in. in cross section by 2 ft. long and were covered with a close mesh artificial silk. One cage consisted of a wire frame over which the material was sewn, leaving a sleeve at one end. The other was more elaborate, being made of a wooden frame, one long side of which had a glass panel and the rest was covered by the material with a sleeve as in the other cage. The two cages were suspended by cord from the ceiling about 5 ft. from the floor, so that ants might be excluded. One half of each cage was covered with brown paper to give conditions of light and darkness, and on the top of each cage a wet towel was laid and was kept damp throughout the experiments. Inside a piece of cotton wool soaked in diluted honey was suspended. In addition to the cages, the method described by Waterston (1922) (Fig. 4, p. 87) for keeping *Phlebotomus* was used. A number of unglazed earthenware flower pots  $3\frac{1}{2}$  in. high by 3 in. in widest diameter were obtained, and were stood in plates of the same material, 5 in. diameter by  $\frac{1}{2}$  in. deep, containing a small quantity of water. The mouth of each pot was covered with a piece of muslin with a sleeve in the middle through which a test tube was inserted and tied vertically mouth downwards about 1 in. from the bottom of the pot. Two to four flies were kept in each test-tube which was closed with a piece of muslin.

Both the cage and pot method proved relatively successful, and although a good many flies died prematurely, some remained alive for as long as fourteen days. The longer span of life resulting from these methods seemed to be due to the conditions of higher humidity and lower temperature which they produced. Temperatures were taken daily in the laboratory during the first week in July and were noted to vary from 27.5 to 29.5° C. in the forenoon (about 10 a.m.) to 28 to 32°C. in the

afternoon (3-4 p.m.). The temperature inside the earthenware pots was usually from about 3 to 6°C. lower than that of the laboratory, but in the cages it was only about 1 to 2°C. lower.

Three batches of fed flies were received on the 26th and 28th June and 1st July respectively. The first batch numbering 27, all of which had had their infective feed from one subject (P.S.) were put in 8 test-tubes in the earthenware pots. The tubes contained 1 to 7 flies and for nourishment a drop of diluted honey was put on the muslin cover and was renewed each day, and also the flies were offered a blood feed but this was only taken by 3 flies on one occasion early in the experiment. The results are seen in the table :—

TABLE II.  
Experimental infection of *Culicoides*. (Pot method.)

Days.	1	2	3	4	5	6	7	8	9	10	11	
Number of flies dying each day after infective feed ... ...	8	5	0	4	0	4	0	2	2	1	1	Total number of flies =27
Number of flies examined ...	0	0	0	4	0	3	0	2	2	1	1	Total examined =13
Number of flies found infected with larvae ...	0	0	0	3	0	2	0	2	1	0	1	Total infected=9

A rather heavy mortality in the 1st and 2nd day was followed by a more gradual one, the longest lived fly being 11 days old. Of the four flies which died on the 4th day, three were infected with larvae in the thorax which were all developed to about the same degree, somewhat in advance of the stumpy "sausage" stage and with alimentary tracts visible. One of these flies, however, had a single apparently infective larva in the head, which was doubtless the result of a natural infection. Of the three flies which died on the sixth day, two were carrying still further advanced larvae in the thorax and head, and thorax, respectively. On the 8th day one of the flies was killed by pressure, and a hot needle was brought close to the head in the hope that if larvae were present they would be stimulated to emerge via the proboscis, but nothing happened. On dissecting it a single larva was found in the head. In the other fly,

which died naturally a similar larva was found in the thorax. The single fly which survived till the 11th day was anaesthetized with chloroform and then beheaded with a pair of dissecting needles. After a wait of two minutes there was no sign of a larva emerging, which was described in the case of *F. persans* after this operation, and gentle pressure was then applied to the head with a needle and a larva slowly moved out through the proboscis. A strong solution of methylene blue was applied when it was about halfway out, which paralysed it, after which it was fixed in situ in 10% formalin.

TABLE III.  
Experimental infection of *Culicoides*. (Cage No. 1.)

Days.	1	2	3	4	5	6	7	8	9	10	11	
Number of flies dying each day after infective feed ... ...		2	3	1	0	2	4	5	1	14		Total number of flies=32
Number of flies examined ...		2	3	1	0	1	4	5	1	12		Total examined =29
Number of flies found infected with larvae ...							2	1	1	1		Total infected=5

The early developing stages of the parasites such as the "sausage" larvae showed little evidence of life on being dissected from the thoracic muscles, but once or twice feeble motions were seen. The later more nematode-like stages in the thorax and head were quite active on disruption from the sand-fly in normal saline and remained thus for as long as about 15 minutes.

The second batch of flies, collected and fed by a second infected case (V.L.) on the evening of 28.vi.33 were received the following morning. These, numbering 32, were put into one of the cages described. The results are seen in Table III.

This method was also successful for on the 11th day nearly 50% of the flies were still living and no doubt would have lived longer but they were killed for examination. In addition to the honey water which was always present in the cage, a blood feed was offered each day, an arm being put into the cage for about half an hour. On the 1st day five flies fed, on the

2nd eight fed, on the 3rd one fed, on the 4th two fed and on the 7th four fed. Thus 20 blood meals were taken during the experiment, but whether by 20 different flies or not it is impossible to say. It is unlikely that 20 different flies fed, for previously a tubed fly had been noted to feed 5 times on 5 successive days. On the other hand it is possible that more than twenty flies fed, for the number was estimated according to the number of bites felt on the arm, and previous experiences showed that sometimes flies could bite and feed and no irritation was felt. It was noticed that the majority of flies showed a preference for light by remaining in the undarkened part of the cages, and a marked positive phototropism was also demonstrated when an electric torch was held to the end of a tube containing several flies. This fact proved useful in the manipulation of live flies, *e.g.*, transferring them from one tube to another and minimised the number of accidental escapes.

Of the five flies found infected in Cage No. 1, two had larvae in the head and three had thoracic infections. The larvae, which were eight days old at the least, were all in the advanced stage previously noted.

The relatively high percentage of infected flies in Table II compared with that of Table III is correlated with the donors of the infective feed. Microfilariae were more numerous in the blood of P.S., who fed the flies in Table II, than in V.L., who in addition was rather an anaemic type.

On the morning of 1.vii.33 a third batch of flies, caught and fed on the previous evening, was received from each subject (P.S. 33 and V.L. 35). They were released into the second cage which was set up in the same way as the first. Of these 68 flies only 36 were finally recovered, partly owing to accidental escapes when the sleeve was opened either to feed the flies or to remove dead ones, and partly due to the accidental introduction of an ant which must have been responsible for the disappearance of several of the missing flies, before its presence was noted. The results obtained from this batch are seen in Table IV.

The larvae found on the 4th day were in the advanced "sausage" stage, whilst those found on the 6th day were similar to the infective stage of the head. There were ten of them in the thorax, which was the largest number found in one fly. On subsequent days the infected flies had larvae in the head only or in the head and thorax. In one of them emergence from the proboscis was again witnessed upon slight pressure with a

needle. Of this series only eight partook of a blood meal during the experiment, viz.: seven on the 1st day and one on the 3rd day.

TABLE IV.  
Experimental infection of *Culicoides*. (Cage No. 2.)

Days	1	2	3	4	5	6	7	8	9	10	11	12	13	
Number of flies dying each day after infective feed...	1	5	4	3	1	1	4	6	5	0	1	2	3	Total number of flies=36
Number of flies examined	0	5	4	3	1	1	4	6	5	0	1	2	3	Total examined =35
Number of flies found infected with larvae	0	0	0	1	0	1	2	2	1	0	0	2	0	Total infected =9

The method of examining flies for infection in the above experiments was to dissect the fly with a pair of needles in a drop of saline on a slide under a low power of the microscope. It was desirable, however, that serial section of infected flies should also be made. Accordingly smaller batches of fed flies, received at different times during the above experiments, were kept alive by the pot method and killed at suitable intervals. They were embedded in celloidin and sectioned. Of 23 flies thus treated, 16 were found to be carrying larvae in the thorax or head. Only one of these appeared to be a natural infection. Some of the results obtained from this series of flies are seen in the photomicrographs. The total results of the experimental infections are summarised in Table V.

Since the flies used in all the experiments were wild flies and might be expected to harbour, and were found to harbour to a small degree, larvae which were obviously the result of a natural infection, it is necessary to consider this fact in connection with the experimental results. At the same time the question of the species of *Culicoides* used in these experiments must be considered. During the work it was not known what species or how many species were being used, but it was thought that more than one species was involved, and with this possibility in view, the wings

of many of the flies used in the experiments were preserved. In this way identification of flies which had already been dissected or sectioned was possible. Subsequent identifications of a collection made at Calliaqua showed that two species were present there, viz.: *C. furens* Poey and *C. paraensis* Goeldi. Of sixty experimental flies identified from preserved wings, 57 proved to be *C. furens* and only three were *C. paraensis*. Thirty of these had been successfully infected and proved to be *C. furens* only. (Whether *C. furens* is the commoner species of the two in Calliaqua, or whether it was more prevalent in the evening when the collecting was done, was not ascertained; but in either of the two possibilities appears to lie the explanation of the predominance of *C. furens* in the experimental flies.) In comparing the percentage of experimental flies harbouring

TABLE V.  
Summary of experimental infections of *Culicoides*.

Days after infective feed.	1	2	3	4	5	6	7	8	9	10	11	12	13	
Number of flies exam- ined ...	14	42	45	32	6	9	5	14	12	2	14	2	3	Total examined = 200
Number of flies found infected with larvae	6	9	6	13	1	4	2	6	3	1	2	2	0	Total infected = 55
														Percentage in- fected = 27.5

larvae with that of wild flies, it seems advisable therefore to consider the natural infection percentage in *C. furens* only. A hundred of this species were collected at Calliaqua and on dissection five of them yielded larvae apparently identical with stages taken from the experimental flies. In one of them the head was infected and in the others the thorax only. The statistical calculation of the difference between the percentage of experimental infections, i.e., 27.5% and that of the natural infections, i.e., 5% is  $22.5 \pm 4.9$ , and the ratio of this difference to its own standard error is thus 4.59, which is highly significant.

MORPHOLOGY OF LARVAL STAGES OF *F. OZZARDI*.

The morphology of the microfilariae as it occurs in the definitive host has been fully described elsewhere and requires no further mention here. Within twenty-four hours after it has been ingested by the sandfly it migrates to the thoracic muscles where it lies stretched out straight between the muscle fibres. By this time it has already slightly changed its form. It is considerably shorter, being only 130–160 $\mu$  long and is showing signs of transformation to the "sausage" stage, that is to say, the body is broadening in the region behind the middle, and then tapers off to a very fine point. (Fig. 2 and Plate VI, Fig. 12.) The internal structure is also undergoing changes. A clear non-nucleated space at the oral end of the larva is still present but the oblique gap representing the nerve ring is very indistinct, as also is the excretory pore space. The anal space is also indistinct. The excretory cell, however, and the G<sub>1</sub>–G<sub>4</sub> cells are clearly visible in the larvae in the stained sandfly sections, and are more easily recognisable than in the microfilaria in the blood. In several instances there was a zone of stained nuclei in the musculature of the sandfly around the posterior half of the larva and somewhat posterior to it, suggesting that a host reaction to the newly arrived parasite is taking place. It was not seen in relation to any of the later larval stages.

The rate of development of the larvae during the next two or three days is very unequal, for at any one time larvae may be found in one fly which are in considerably different degrees of development. Without increasing in length the 24 hours larva becomes fatter, about four times the diameter of the microfilaria, remains rounded at the oral end and retains a sharply pointed tail, 10–20 $\mu$  long, at the posterior end. In the

Fig. 1. *Microfilaria ozzardi* in blood.

Figs. 2 & 3. Larvae from thorax of sandfly 1 day after infective feed.

„ 4 to 6. 1st stage or "sausage" larvae from thorax, 3 days after infective feed.

„ 7 & 8. Larvae 3 days after infective feed, with alimentary tract, vacuoles, excretory pore and oral stylets.

„ 9 & 10. Larvae 4 days after infective feed, with caudal papillae of 2nd stage. Fig. 10. The tail of the 1st stage larva is about to be cast off in an ecdysis.

Fig. 11. 2nd stage larva from thorax, 4 days after infective feed.

„ 12. 3rd stage larva from thorax, 7 days after infective feed. The larva is still enclosed in the sheath of the 2nd stage, and is about to cast it off.

„ 13. Infective 3rd stage larva from head of sandfly, 8 days after infective feed. (Cytological details not drawn in Figs. 9 to 13.)



unstained state its contents are vacuolar and granular and as yet no signs of an alimentary canal are visible (Fig. 4). At this time the maximum diameter may be in the latter half of the body and there may be a slight narrowing in the middle (Fig. 5). Growth proceeds and with it internal development is also rapidly taking place. In a larva about 72 hours after ingestion the alimentary canal is apparent and is composed of a tube whose wall is one cell thick stretching from mouth to subterminal anus. Its cellular nature makes it clearly visible for the posterior half or two-thirds, but anteriorly it is not so clear and the mouth-opening is very indistinct. An excretory pore in the anterior quarter is a definite feature at this stage and with it is associated a large sperical vacuole occupying nearly the width of the body. A similar vacuole is situated near the anus and tends to obscure a rectal sac which connects the anus and intestine. The vacuoles in the living larvae are very striking as they are filled with a highly refractive fluid. In several larvae of about this age a pair of highly refractive objects were seen at the oral aperture. Apparently of a hard chitinous material and somewhat triangular in shape it is possible that they represent oral stylets. (They have been figured by Davis (1928) in a larva found in the thorax of *Anopheles tarsimaculatus* infected 12 days previously and Manson (1884) has referred to similar objects as papillae in a larva of *F. bancrofti* taken from a mosquito 152 hours after feeding.)

The first ecdysis takes place about the 3rd or 4th day, and results in the loss of the short spiked tail or "sickle" of Manson's *F. bancrofti* larva. The shedding of the cuticle reveals morphological changes which have been going on for some time previously. The oral aperture is now apparent and the tail is blunt and papillated. There are two pairs of lateral papillae, the anterior pair of which are pedunculate and situated about  $10\mu$  from the tip of the tail. The other pair are sub-terminal and more stumpy, while the tip of the tail is bluntly conical (Fig. 16). This larva, characterised by a papillated tail and oral stylets or papillae, is the 2nd stage larva in the accepted nematode sense of the term, since only one ecdysis has preceded it. The 1st stage larva is distinguished from it externally by its smaller size and spiked tail, but it is difficult to find any internal morphological character which distinguishes the 1st and 2nd stage larvae. It is probable that the development of an alimentary tract is a manifestation of the change from 1st to 2nd stage, for during this process the spiked tail is becoming less and less conspicuous until it is little more

than an empty sheath. Although the moulting was not actually observed, the 2nd stage larva just before or soon after the cuticle is cast is about 0.3 to 0.4 mm. long by 0.03 to 0.02 mm. in width.

During the 5th and 6th days this larva becomes longer and thinner; further changes in morphology are occurring and a second ecdysis is being prepared. A six-day old larva dissected from the thorax measured 0.67 mm. long by 0.25 mm. in width and was in the process of ecdysis, for at the tail end the cuticle could be plainly seen coming away from the body (Fig. 12) and through it the tail of the 3rd stage larvae was visible. It was truncated and quite unlike that of the 2nd or 1st stage larva.

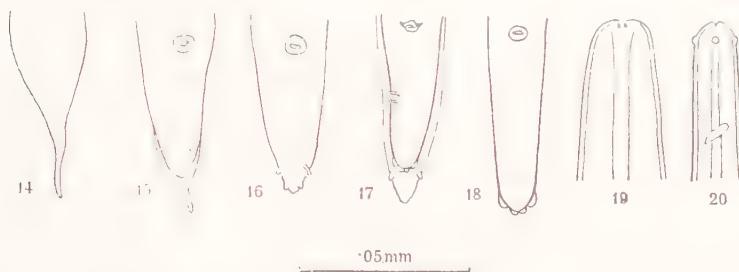


Fig. 14. Tail of "sausage" or 1st stage larva.  
,, 15. Tail of 2nd stage larva in sheath.  
,, 16. Tail of 2nd stage larva.  
,, 17. Tail of 3rd stage larva in sheath.  
,, 18. Tail of 3rd stage larva.  
,, 19. Oral end of 2nd stage larva.  
,, 20. Oral end of 3rd stage larva.

Very close to the tip are a pair of lateral lobes or large papillae, and the terminal part of the tail between them is distinctly bifid. On the sheath itself the cast of the two anterior papillae of the 2nd stage were visible, and to the sheath at the anal aperture a cuticular fragment of the rectum of the 2nd stage larvae was adhering. Of the same age and in the thorax of the same sandfly were two other larvae which had completely exsheathed and represented true 3rd stage larvae. These were a little longer and considerably thinner than the larva just described, being over 0.7 mm. and a little less than 0.02 mm. wide. Almost cylindrical throughout they taper very gradually in the posterior quarter to the anus about 0.05 mm. from the tip, beyond which the tail narrows rather more abruptly to the lobate extremity already described. Anteriorly there is a gradual tapering from about 0.05 mm. to the oral end which is rounded.

Lips could not be seen surrounding the mouth, but four papillae, arranged laterally and dorso-ventrally are prominent features (Fig. 20). The alimentary canal is well-defined but differentiation into oesophagus and intestine was not noted and the cellular structure of this organ so obvious in the early 2nd stage larvae is no longer evident. The anus is situated on a slight prominence. Genital cells appear to be as yet undeveloped, and the excretory pore and nerve ring are very difficult to discern. The cuticle is very stout and perfectly smooth. Owing to their length the 3rd stage larvae are coiled or bent on themselves in the thorax of the fly and none of the sections show a complete larva. In the head also they are much cramped and in this site were never found more than two in number, usually with part of the body in the head and part in the proboscis or else extending from the head into the thorax. The longest 3rd stage larva found was dissected from the head and measured 0·78 mm. in length.

No further changes in morphology were observed even in larvae which had been in a sandfly as long as 13 days and it can be assumed on morphological and biological grounds that this 3rd stage larvae is the infective stage. Owing to the scarcity of material it was not possible to observe the final process in the intermediate phase of this life-cycle by attempting a human infection or possibly by observing skin penetration of some experimental animal by the infective larva.

#### DISCUSSION.

The main points in the development of *F. ozzardi* in its intermediate host resemble, with certain interesting exceptions, those described in the other four human filarial worms. Soon after being ingested by the vector the microfilaria undergoes the typical shortening and exaggerated increase in breadth which gave rise to the term "sausage" larva, which larva is also characterised by a short spiked tail. Then follows elongation together with the development of an alimentary canal which is followed by an ecdysis and the loss of the spiked tail. Further elongation and a marked decrease in the width of the body is followed by another ecdysis which results in the final or infective stage. By the interpolation of *two* ecdysis in its development the larva of *F. ozzardi* appears to differ from the other human filariae. In the case of *F. bancrofti* there appears at first sight, according to Manson's description and figures, to be only one ecdysis. (The exsheathment of the microfilaria in the stomach

of the mosquito, if the theory be correct that this sheath is merely a stretched-out egg-shell, is not a true ecdysis.) Manson regarded the disappearance of the spiked tail of the sausage larva as probably due to its being cast off in a general ecdysis. A study of his drawings, however, reveals furthermore that a second ecdysis has taken place before the final stage, characterised by a trilobed tail, has been attained. This is apparent both in his Fig. 31 and 46. In the latter two sheaths are seen extending beyond the end of the tail which is assuming the trilobed form, and naturally this implied two ecdyses. In his Fig. 31 the trilobed tail is further developed and is enclosed in a single sheath which does not terminate in a spike but is round and therefore must represent a second ecdysis. It is probable that in Fig. 29 the 2nd stage larva is represented. In any case it is evident, if this interpretation be correct, that the 2nd stage is not well defined and of short duration.

In the case of the development of *Loa loa*, as described by Connal and Connal (1922), there is, excluding the exsheathment of the microfilaria, but one ecdysis. This results in the loss of the spiked tail as in *F. bancrofti* and *F. ozzardi*, but is said to reveal a larva with a trilobed tail. In the life-cycle of *Onchocerca volvulus*, Blacklock (1926) only observed one ecdysis, viz.:—"in the case of the transition from the tailed thorax form to the thoracic form without tail." He suggests, however, that later another ecdysis may have occurred. Sharp (1927) described, in the development of *F. perstans* in sand-flies, an ecdysis which occurred within six hours after the microfilaria was ingested by the fly, and states "it is probable that one and possibly three more occur before the larva reaches the proboscis." The biological significance of this is difficult to estimate since, of course, the microfilaria of *F. perstans* is sheathless and such an early ecdysis was hitherto only known to occur in the case of the sheathed microfilariae of *F. bancrofti* and *Loa loa*, but possibly it can be attributed to the sudden shortening of the microfilaria after it has entered the intermediate host. A similar sudden shortening was also noted to have taken place in the microfilariae of *F. ozzardi* but unfortunately an ecdysis was not observed.

The position then with regard to the larval development of the human filariae is an interesting one. From a study of the majority of the known life-cycles of parasitic nematodes where existence outside the definitive host depends upon a parasitic sojourn in an intermediate host or upon a

free-living phase in the soil, it appears that during this time the metamorphoses of such larvae involve two ecdyses. These ecdyses bring the larva to a stage which is the infective one for the definitive host. In the Spiruroidea, a group which has affinities with the Filarioidea, several life-cycles can be cited in which this takes place. In *Spirocercus sanguinolenta* and *Phyocephalus sexalatus* from the dog and pig respectively, the larval development is enacted in the bodies of coprophagous insects, in which the two ecdyses occur which produce an infective 3rd stage larva. In a species probably more closely allied to filariae, viz.:—*Dracunculus medinensis*, two ecdyses also occur in the body of the intermediate host *Cyclops*. In many of the bursate nematodes the same thing happens during the non-parasitic phase in the soil, which results in an infective 3rd stage larva, the classical example of which is seen in the hookworm life-cycle. It is apparent then, that in two at least of the human filarial worms, the larval development in an insect intermediate host conforms with what is a common and widespread phenomenon amongst nematodes.

#### SUMMARY.

1. The life-cycle of *Filaria ozzardi* was studied in St. Vincent, B.W.I.
2. *Culicoides furens* Poey, a common sandfly in Calliaqua, where a 37.7% human infection was found, was used in the experimental infections.
3. Two hundred sand-flies, collected at Calliaqua, were given an infective feed of blood of *F. ozzardi* carriers. Of these flies, fifty-five, i.e., 27.5%, were subsequently found to be infected with developing stages of the parasite. The ingested microfilariae migrated within 24 hours to the thorax, where the entire morphological development takes place. In flies which were kept alive for seven or eight days, advanced stages were found in the thorax and head, and their emergence from the proboscis was induced by slight pressure on the head.
4. The development in the sandfly consists of a metamorphosis from the 1st stage or "sausage" larva to a 2nd stage larva during the first 3 to 4 days, and from this stage to a 3rd stage or infective larva on the fifth or sixth day. Two ecdyses occur during these metamorphoses, which are discussed in relation to the other human filarial parasites.

5. Five per cent. of *C. furens* caught at Calliaqua were found to be naturally infected with developing larvae, apparently *F. ozzardi*.

6. The possibility that *C. paraensis*, another species in Calliaqua, may also act as a vector, is not excluded.

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## PLATE I.

Fig. 1. *Culicoides furens* Poey. Adult female.  $\times 35$ .  
,, 2. *Microfilaria ozzardi* in blood.  $\times 500$ .

## PLATE II.

Fig. 3. Larvae one day old, in longitudinal muscles.  $\times 120$ .  
,, 4. Larvae two days old, in vertical thoracic muscles.  $\times 120$ .

## PLATE III.

Fig. 5. Larvae four days old, in vertical thoracic muscles.  $\times 120$ .  
,, 6. Larvae seven days old, in thorax of *C. furens*.  $\times 120$ .

## PLATE IV.

Fig. 7. Larvae seven days old, in thorax of *C. furens*.  $\times 120$ .  
,, 8. Larvae seven days old, passing from thorax into head.  $\times 120$ .

## PLATE V.

Fig. 9. Larva in head of *C. furens*.  $\times 235$ .  
,, 10. Larva emerging from proboscis, eight days old.  $\times 90$ .

PLATE VI  $\times 235$ .

Fig. 11. *Microfilaria ozzardi* in blood. Fig. 12. Larva from thorax, one day old. Fig. 13. Larvae from thorax, three days old. Figs. 14 & 15. Ensheathed larvae from thorax, four days old. Fig. 16. Larva (2nd stage) from thorax, four days old. Fig. 17. Larva (3rd stage) from thorax, seven days old. Fig. 18. Larva (3rd stage) from head, eight days old.

## PLATES VII &amp; VIII.

Calliaqua, St. Vincent, B.W.I.

J. J. C. BUCKLEY

Development of *F. ozzardi* in *C. furens*



PLATE I

J. J. C. BUCKLEY  
Development of *F. ozzardi* in *C. fitrens.*



PLATE II.

J. J. C. BUCKLEY

Development of *F. ozzardi* in *C. furcans*.

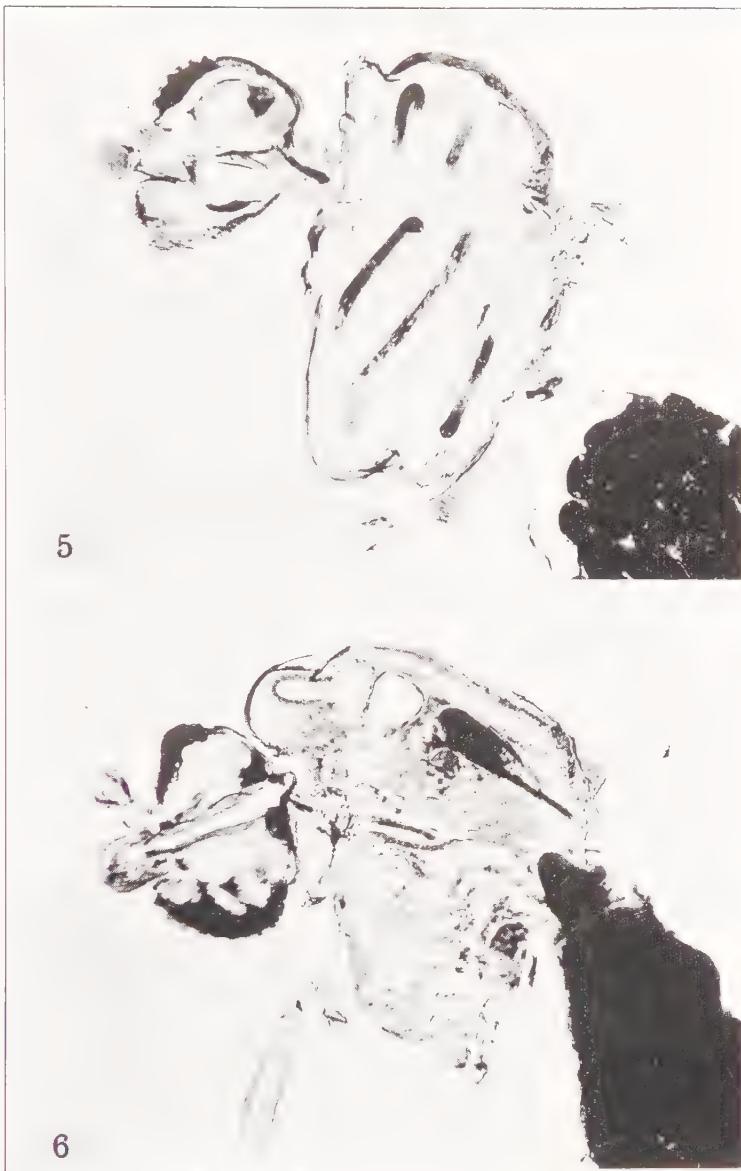


PLATE III.

J. J. C. BUCKLEY

Development of *F. ozzardi* in *C. furens*.



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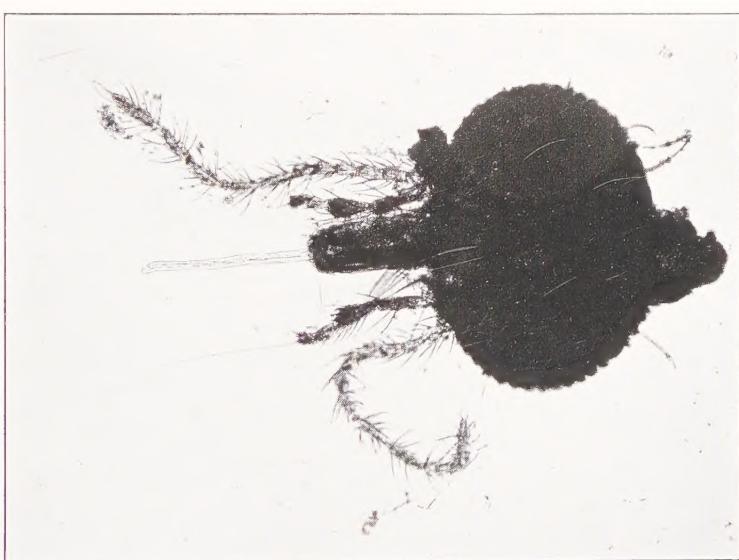


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PLATE IV

J. J. C. BUCKLEY

Development of *F. ozzardi* in *C. furens*.



J. J. C. BUCKLEY

Development of *F. ozzardi* in *C. furens*.

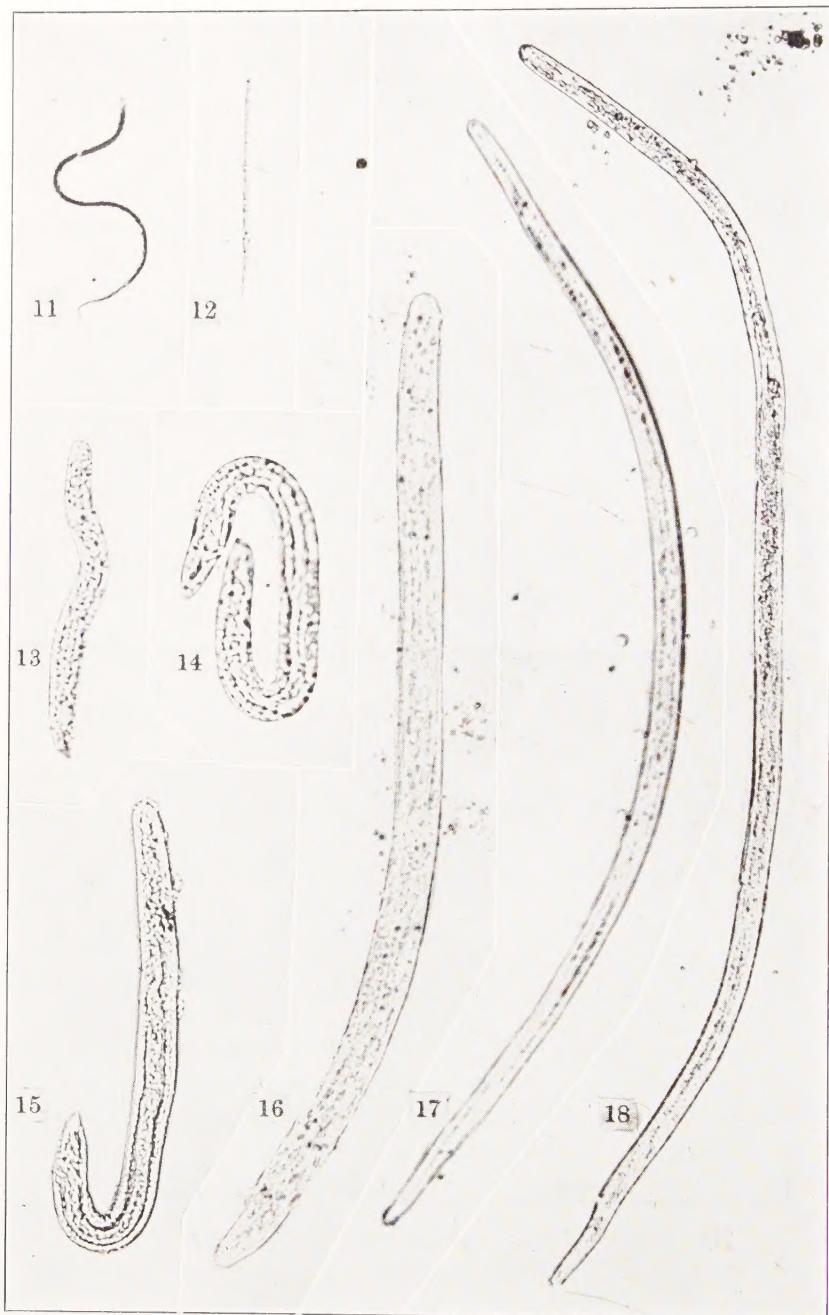
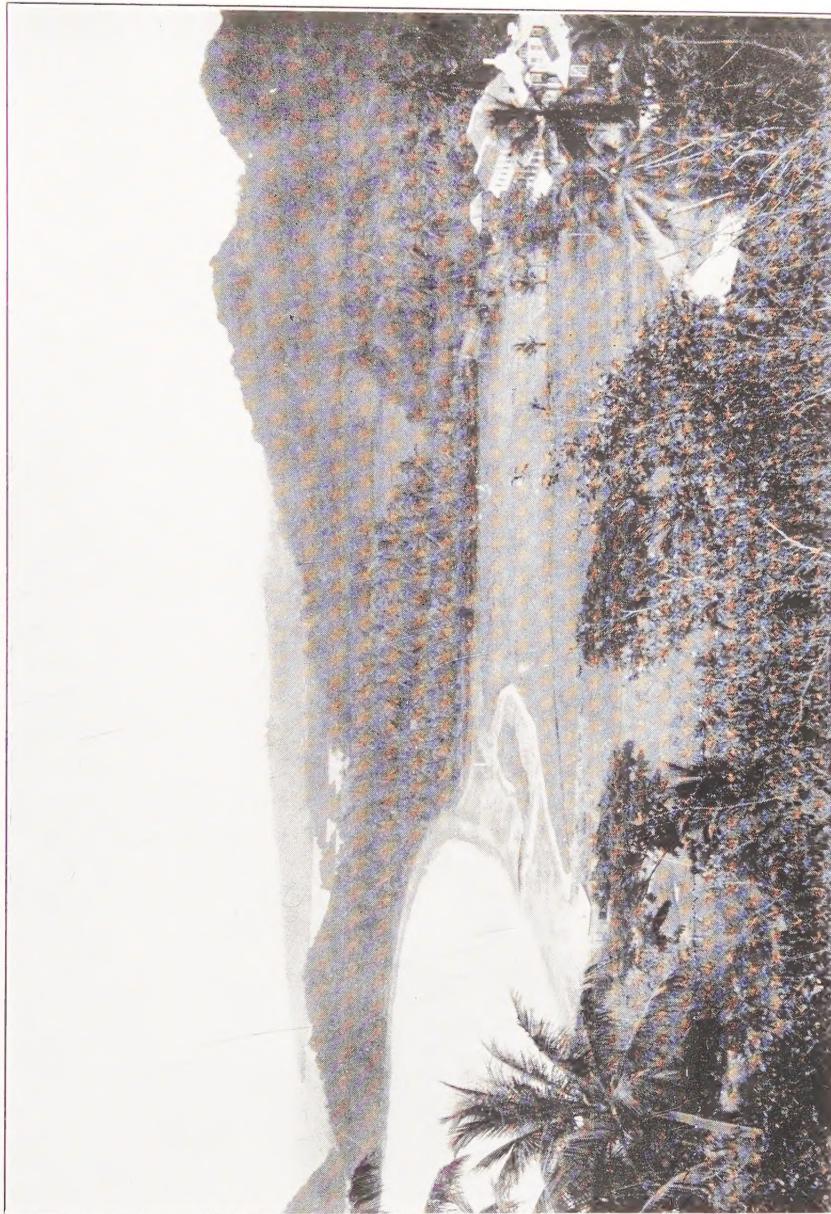


PLATE VI.

PLATE VII.



Callqua, St. Vincent, B.W.I.

PLATE VIII.



Calliaqua, St. Vincent, B.W.I.